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**THE EFFECTS OF EXERCISE-INDUCED MUSCLE DAMAGE ON
ENDURANCE PERFORMANCE**

**This thesis is submitted in accordance with the requirements of the University
of Chester for the degree of Doctor of Philosophy**

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Abstract

It is well documented that engaging in resistance exercise can lead to further improvements in endurance performance. Whilst, not fully understood, it is speculated that increased motor unit recruitment, improved muscle coordination and enhanced utilisation of stored elastic energy after resistance-based exercise improves exercise economy. Nevertheless, while prolonged exposure to resistance training improves endurance performance in the long-term, a consequence of such training when unaccustomed is the appearance of exercise-induced muscle damage (EIMD). Exercise-induced muscle damage is well known to affect athletic performance requiring muscular strength and power; however, its effects on markers of endurance exercise are unclear. Therefore, the aim of this thesis was to investigate the effects of EIMD on endurance performance, with an emphasis on the physiological (oxygen uptake; $\dot{V}O_2$, minute ventilation; \dot{V}_E), metabolic (blood lactate; [La]), perceptual (rating of perceived exertion; RPE) and kinematic (stride length; SL, stride frequency; SF) responses during sub-maximal endurance exercise.

STUDY 1 (CHAPTER 3)

INTRODUCTION: A possible explanation for the equivocal findings of EIMD on endurance performance is that responses appear to be sensitive to the mode of endurance exercise adopted. Research examining the effects of EIMD on cycling have observed no change in sub-maximal oxygen cost, conversely, research investigating running responses to EIMD have reported increases. Study 1 examined the influence of exercise mode (cycling versus running) on physiological, metabolic, perceptual and kinematic responses after EIMD. **METHODS:** Ten male participants performed counter-balanced incremental cycling and running trials to exhaustion to

establish lactate threshold (LT) for each mode. Participants then completed two counter-balanced 10 min cycling and running bouts at LT before, 24 h and 48 h after muscle-damaging exercise. $\dot{V}O_2$, \dot{V}_E , RPE, running SL and SF and cycling revolutions per minute (RPM) were measured throughout each exercise bout, whilst [La] was recorded before and at the end of each exercise bout. Perceived levels of muscle soreness, peak isokinetic knee extensor torque at 60 deg·s⁻¹ and creatine kinase (CK) activity measured at baseline, 24 h and 48 h were used to indicate the presence of muscle damage. **RESULTS:** The squatting exercise was effective ($P < 0.05$) in causing increased muscle soreness (*Baseline*: 0.4 ± 0.5 ; *24 h*: 5.4 ± 1.5 ; *48 h*: 6.7 ± 1.3), elevated CK activity (*Baseline*: 78.1 ± 30.4 U·l⁻¹; *24 h*: 238.9 ± 181.6 U·l⁻¹; *48 h*: 143.1 ± 83.9 U·l⁻¹) and decreased knee extensor strength (by 13.6 – 14.9%) at 24 and 48 h. Increases in $\dot{V}O_2$ and \dot{V}_E were observed during both cycling and running after EIMD. However, the time course of these appeared to be mode specific, with increases in $\dot{V}O_2$ (by 3.5 – 3.7%) and \dot{V}_E (by 9 – 11.3%) during running occurring at 24 and 48 h after EIMD, while responses during cycling were only elevated at 48 h ($\dot{V}O_2$ and \dot{V}_E increased by 5.3% and 12.3% respectively). RPE was increased during both modes after EIMD, [La] responses remained unchanged, SL and SF were altered during running, whilst cycling RPM was unchanged. **CONCLUSIONS:** This is the first study to examine the same bout of EIMD on responses to sub-maximal running and cycling exercise. The results demonstrate that physiological responses are increased during both cycling and running after EIMD. Nevertheless, it was demonstrated that the time course of these responses are mode specific. The increased $\dot{V}O_2$ response during running is attributed to changes in stride pattern, while the unexpected increased $\dot{V}O_2$ cycling response

might be due to the recruitment of auxiliary muscles after EIMD. It is postulated that the differences in ventilation between exercise modes are due to different stimuli activating afferent fibres post-EIMD. Individuals considering resistance training to aid endurance performance should be aware of the consequences that unaccustomed resistance exercise has on endurance exercise performed in the ensuing days.

STUDY 2 (CHAPTER 4)

INTRODUCTION: Findings from Study 1 confirm that EIMD increases oxygen uptake during endurance exercise. Furthermore, previous research has shown muscle-damaging exercise to increase oxidative metabolism at rest. Despite this, it is not known whether oxygen uptake during recovery from endurance exercise is increased when experiencing EIMD. Therefore, the aim of Study 2 was to investigate the effects of EIMD on $\dot{V}O_2$ before, during and after sub-maximal running.

METHODS: Eight healthy male participants performed an incremental running test to volitional exhaustion to determine lactate turn-point (LTP) and $\dot{V}O_{2\ peak}$. After a 12 h fast, participants completed baseline measurements comprising resting metabolic rate (RMR), indirect markers of EIMD, 10 minutes of sub-maximal running and 30 minutes of recovery to ascertain their excess post-exercise oxygen consumption (EPOC). Measurements were then repeated at 24 and 48 h after 100 Smith-machine squats. **RESULTS:** Data analysis revealed significant increases in ratings of muscle soreness (*Baseline*: 0.2 ± 0.5 ; *24 h*: 6 ± 1.6 ; *48 h*: 6.6 ± 2.1) and CK (*Baseline*: $37.3 \pm 47.1 \text{ U}\cdot\text{l}^{-1}$; *24 h*: $125.7 \pm 69 \text{ U}\cdot\text{l}^{-1}$; *48 h*: $78.9 \pm 36.4 \text{ U}\cdot\text{l}^{-1}$) and decreases in peak knee extensor torque (by 20 – 22%) at 24 and 48 h after squatting exercise. Moreover, RMR (by 11.8 – 13.2%), $\dot{V}O_2$ during sub-maximal running (by 4 – 5%) and EPOC (by 4 – 6%) were increased in the two days after squatting exercise.

CONCLUSIONS: This is the first study to demonstrate that in the presence of EIMD, oxygen uptake is increased at rest, during and after sub-maximal running. It is suggested that the elevated RMR was a consequence of a raised energy requirement for the degradation and resynthesis of damaged muscle fibres. The increased oxygen demand during sub-maximal running after muscle damage was responsible for the increase in EPOC. Individuals engaging in unaccustomed resistance exercise that results in muscle damage should be mindful of the increases in resting energy expenditure and increased metabolic demand to exercise in the days that follow.

STUDY 3 (CHAPTER 5)

INTRODUCTION: The results from Studies 1 and 2 inform that EIMD has a detrimental effect on endurance exercise performed in the days that follow. However, it is unknown whether such effects remain after a repeated bout of EIMD. Therefore, the purpose of Study 3 was to examine the effects of repeated bouts of muscle-damaging exercise on sub-maximal running exercise. **METHODS:** Nine male participants performed an exhaustive incremental running trial to establish LTP and $\dot{V}O_{2peak}$. Participants then completed baseline measurements of perceived muscle soreness, peak isokinetic knee extensor torque, CK and vertical jump performance. Additionally, a 10 min running bout at individual LTP was performed, with $\dot{V}O_2$, \dot{V}_E , [La], RPE, SL and SF recorded throughout. These measurements were then repeated 24 and 48 h after squatting muscle-damaging exercise. Two weeks later, when the symptoms associated with the initial bout of EIMD had dissipated, baseline procedures, the same muscle-damaging exercise and follow-up testing were repeated. **RESULTS:** Data analysis revealed significant changes in the indirect

markers of muscle damage at 24 and 48 h after the initial bout of EIMD, but after the repeated bout, the symptoms of EIMD were attenuated. Moreover, the significant changes (relative to baseline) in $\dot{V}O_2$, \dot{V}_E , [La], RPE, SL and SF evident at 24 and 48 h after the initial bout of EIMD were absent after the repeated bout of EIMD.

CONCLUSIONS: In agreement with findings from Studies 1 and 2, this study demonstrated that the physiological, metabolic, perceptual and kinematic responses during endurance exercise were altered after an initial bout EIMD. However, for the first time, Study 3 shows that the detrimental effects of EIMD on endurance exercise are negated after a repeated bout of EIMD. This protective adaptation is known as the repeated bout effect (RBE) and is attributed to an interaction of various neural and peripheral mechanisms. Individuals should be aware of the negative effects unaccustomed resistance exercise has on endurance exercise performed in the days that follow. Thereafter, as this study informs, less recovery will be required in the days after a repeated bout of resistance exercise.

STUDY 4 (CHAPTER 6)

INTRODUCTION: As shown in Study 3, the effects of EIMD on fixed-intensity endurance exercise are attenuated after a repeated bout of muscle-damaging exercise performed two weeks later. Accordingly, Study 4 examined if a low volume bout of muscle-damaging exercise performed two weeks prior to a high volume bout of the same exercise provided the same protective effect on endurance running exercise. **METHODS:** Sixteen male participants were randomly assigned to a low volume (n = 8) or high volume (n = 8) muscle damage group and completed baseline measurements associated with fixed-intensity and 3 km time-trial running. Measurements were repeated 24 and 48 h (except the time-trial at 24 h) after EIMD,

comprising low volume (50) or high (100) volume squats. Two weeks later, participants repeated the baseline measurements, high volume squats and the same follow-up testing. **RESULTS:** Analysis revealed increases in muscle soreness and creatine kinase and decreases in peak knee extensor torque (by 10.4 – 17.1%) 24 and 48 h after the initial bouts of EIMD. Alterations in $\dot{V}O_2$ (by 3.8 – 5.5%), \dot{V}_E (by 9.4 – 12.8%), [La] (by 6.3 – 15.8%), RPE (by 9.5 – 13.8%), SL (by 2.5 – 4.1%) and SF (by 2.8 – 4.1%) were found during fixed-intensity running at 24 and 48 h after EIMD. Likewise, time increased (by 7 – 9%) and speed (by 5.4 – 8.3%), $\dot{V}O_2$ (by 7.2 – 9.8%) and [La] (by 19.6 – 21%) all decreased during a 3 km running time-trial 48 h after muscle-damaging exercise. However, symptoms of muscle damage, responses during fixed-intensity running and time-trial performance were attenuated in the days after the repeated bout of high volume EIMD. Furthermore, this adaptation was independent of whether the RBE was preceded by an initial bout of low or high volume EIMD. **CONCLUSIONS:** In agreement with Study 3, this study reaffirms that a single bout of EIMD protects the muscle against the effects of muscle damage on fixed-intensity running. Moreover, for this first time, this study demonstrates that the protective effect of low volume muscle-damaging exercise against high volume EIMD is transferable to endurance running. Furthermore, time-trial performance is found to be preserved after a repeated bout of EIMD. Individuals contemplating concurrent resistance and endurance exercise to improve performance should perform low volume resistance exercise two weeks before engaging in concurrent training to precondition the muscle to tolerate higher volumes of EIMD and its negative effects on endurance performance.

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List of Abbreviations

ACE	Angiotensin conversting enzyme
ACTN3	Alpha-actinin 3
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
Ca ²⁺	Calcium ions
CCL2	Chemokine ligand 2
CK	Creatine kinase
CKMM	Creatine kinase muscle isoform
CMJ	Countermovement jump
CNS	Central nervous system
DJ	Drop jump
DOMS	Delayed onset muscle soreness
E-C	Excitation-contraction
EIMD	Exercise-induced muscle damage
EMD	Electromechanical delay
EMG	Electromyography
f_R	Breathing frequency
GET	Gas exchange threshold
GTO	Golgi tendon organ
h	Hours
HR	Heart rate
HR _{peak}	Peak heart rate
HSP	Heat shock proteins

IGF2	Insulin-like growth factor II
IL	Interleukin
[La]	Blood lactate concentration
LFF	Low-frequency fatigue
LT	Lactate threshold
LTP	Lactate turn-point
MF	Median frequency
MHC	Myosin heavy chains
MLCK	Myosin light chain kinase
MRCP	Movement-related cortical potential
MVC	Maximal voluntary contraction
NIRS	Near infrared spectroscopy
RBE	Repeated bout effect
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
RPM	Revolutions per minute
RQ	Respiratory quotient
SD	Standard deviation
SJ	Squat jump
SR	Sarcoplasmic reticulum
SSC	Stretch-shortening cycle
sTnI	Skeletal troponin I
TNF α	tumour necrosis factor- α

VAS	Visual analogue scale
\dot{V}_E	Minute ventilation
$\dot{V}_E / \dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}_E / \dot{V}O_2$	Ventilatory equivalent for oxygen
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2\max}$	Maximal oxygen uptake
$\dot{V}O_{2\text{peak}}$	Peak oxygen uptake
WBC	White blood cells

CHAPTER 1

INTRODUCTION

1.1 Characteristics of eccentric muscle actions

Muscular contraction is achieved when force is applied against a surface or object. When this force is equal to a load exerted on the muscle, the muscle length remains stationary and an isometric contraction is executed. However, when muscular force is greater than the load exerted, the muscle shortens and a concentric action is performed. Conversely, when this force is lower than that exerted load, the muscle lengthens and an eccentric contraction occurs (Enoka, 1996; Lindstedt et al., 2001).

Eccentric muscle actions possess a number of unique physiological and mechanical attributes compared to concentric actions (Brughelli & Cronin, 2007; Roig et al., 2008a). For example, due to a greater utilisation of type II fibres (McHugh et al., 2002) and greater force production for a given velocity (Allen, 2001), the muscle is capable of producing higher forces during eccentric compared to concentric contractions (LaStayo et al., 1999). These characteristics have implications for strength development, with several studies reporting greater increases in strength after eccentric training compared to concentric resistance training (Komi & Buskirk, 1972; Johnson et al., 1976; Hather et al., 1991; Hortobagyi et al., 1996a; LaStayo et al., 1999; Roig et al., 2008a; Mueller et al., 2009).

Electromyography (EMG) has also shown that for a given force, less motor unit activation is required during eccentric actions when compared against concentric actions (Enoka, 1996; Kellis & Baltzopoulos, 1998; McHugh et al., 2000). This explains why, when eccentric and concentric activity is performed at the same workload, the metabolic cost of eccentric exercise is considerably lower (LaStayo et

al., 1999; Vallejo et al., 2006; Roig et al., 2008b). Bigland-Ritchie and Woods (1976) reported that the oxygen cost of eccentric cycling was ~15% lower than that required during concentric cycling at the same workload. The lower metabolic cost makes eccentric exercise attractive to individuals with a low exercise tolerance or at risk of cardiovascular disease (Lindstedt et al., 2001). In addition, studies have also shown that eccentric exercise increases muscular strength in the elderly (LaStayo et al., 2003) and in patients with chronic conditions, such as obstructive pulmonary disease and heart failure (Roig et al., 2008b).

However, eccentric activity rarely occurs in isolation. During normal human movements, such as running, jumping, throwing and lifting, the muscle contracts in a repeated sequence of eccentric and concentric muscle actions known as the stretch-shortening cycle (SSC) (Komi, 1984; Enoka, 1996). During the SSC, concentric contractions provide the force necessary for propulsive movements to occur. However, it is during the eccentric action that the stretched components of the muscle-tendon system absorb elastic energy that can be used to enhance the force and power produced during the subsequent concentric action (Enoka, 1996; Komi, 2000; LaStayo et al., 2003; Roig et al., 2008a). It is posited that the stiffness of the muscle-tendon system determines the body's ability to utilise elastic energy (Bonacci et al., 2009). Increasing muscle-tendon stiffness has the potential to enhance the storage and utilisation of elastic energy and has been shown to increase power production during vertical jump performance (Lindstedt et al., 2001; 2002; LaStayo et al., 2003). Likewise, running economy, defined as the volume of oxygen required for a given exercise intensity (Jung, 2003), is improved by increasing the stiffness of the muscle-tendon unit (Spurrs et al., 2003). The greater storage of elastic energy during the eccentric component of running increases the force production of the muscle

without reliance on energy from oxidative or glycolytic sources (Saunders et al., 2006; see section 2.1).

1.2 Exercise-induced muscle damage

Eccentric actions are characterised by a 'loading profile' that combines high force with low muscle fibre recruitment (Enoka, 1996; Byrne et al., 2004; Falvo & Bloomer, 2006). The number of actin and myosin cross-bridges within the sarcomere also decrease as the muscle fibre is forced to lengthen, thus resulting in an increased force per cross-bridge (Stauber, 1989; Armstrong et al., 1991; Tee et al., 2007). Furthermore, during eccentric contractions cross-bridges are forcibly detached without requiring adenosine tri-phosphate (ATP) (Enoka, 1996; Byrne et al., 2004). These characteristics impose substantial mechanical stress on the muscle that when repeated during an activity with a high eccentric component can result in EIMD (Byrne et al., 2004; Eston et al., 2004). Exercise-induced muscle damage is a phenomenon that commonly occurs after participation in strenuous, novel or unaccustomed activity. Indeed, eccentric contractions are known to cause symptoms of EIMD with a greater magnitude than those exhibited after isometric or concentric contractions (Komi & Viitasalo, 1977; Newham et al., 1983; Jones et al., 1989). Many activities involve eccentric muscle actions, with symptoms associated with EIMD commonly reported after distance running (Sherman et al., 1984; Kyrolainen et al., 2000), downhill running (Braun & Dutto, 2003; Chen et al., 2009), plyometrics (Nicol et al., 1996; Twist & Eston, 2005; Burt & Twist, 2011), resistance training (Paul et al., 1989; Byrne & Eston, 2002a), bench stepping (Gleeson et al., 1995; Schneider et al., 2007), intermittent shuttle running (Thompson et al., 1999; Magalhaes et al., 2010; Howatson & Milak, 2009) isokinetic knee extensor exercise (McHugh et al., 2001;

Paschalis et al., 2005), team sports (Takarada, 2003; Twist et al., 2012) and occupational and domestic activities (such as lifting tools or the laundry basket; (Paschalis et al., 2011).

The symptoms of EIMD are well documented and include disruption to the intracellular muscular structure and extracellular matrix, delayed onset muscle soreness (DOMS), acute inflammation, muscle stiffness, decreases in range of motion, appearance of myofibre proteins in the bloodstream and the impairment of muscle function (Kendall & Eston, 2002; Byrne et al., 2004; Eston et al., 2004). Typically, these symptoms are exacerbated immediately after a bout of muscle-damaging exercise and can last for several days, depending on the intensity and duration of the exercise adopted (Howatson & van Someren, 2008). Nevertheless, it should be recognised that after unaccustomed muscle-damaging exercise, the muscle undergoes an adaptation that enables it to be more resistant to repeated bouts of EIMD. This protective mechanism is commonly known as the 'repeated bout effect (RBE)' and is typically characterised through a smaller deficit in muscle strength, a lowered perception of DOMS and a reduction of CK in the bloodstream (McHugh et al., 1999a; McHugh, 2003).

1.3 The effects of exercise-induced muscle damage on athletic performance

The detrimental effects of EIMD on measures of athletic performance requiring muscular strength and power are well documented. Studies have shown immediate and prolonged impairments in isometric and isokinetic strength (Byrne et al., 2001; Sayers & Clarkson, 2001; Michaut et al., 2002), peak power output and time to achieve peak power (Sargeant & Dolan, 1987; Byrne & Eston, 2002b; Twist & Eston, 2007), vertical jump performance (Avela et al., 1999; Horita et al., 1999; Byrne &

Eston, 2002a), single and repeated sprint performance during cycling and running (Twist & Eston, 2005; Highton et al., 2009) and agility (Highton et al., 2009) in the days after muscle-damaging exercise. Exercise-induced muscle damage has also been shown to impair endurance performance (Braun & Dutto, 2003; Twist & Eston, 2009; Burt & Twist, 2011), however, the findings appear to be influenced by the methods used to cause muscle damage, the mode of endurance exercise adopted, the exercise intensity and the training status of participants.

1.4 Aims of the current research

The main objective of this thesis was to investigate the effects of EIMD on the physiological, metabolic, perceptual and kinematic responses during endurance performance in recreationally active males.

Aims of Study 1 (Chapter 3)

Changes in the physiological responses during endurance exercise after EIMD appear equivocal. While studies using cycling have observed no change in the sub-maximal oxygen cost after EIMD (Davies et al., 2009; Twist & Eston, 2009), studies using running have tended to report increases (Braun & Dutto, 2003; Chen et al., 2007b). It is suggested that alterations in stride pattern are responsible for the increase in $\dot{V}O_2$ during running after EIMD (Braun & Dutto, 2003). However, to date, no study has examined the physiological responses to the same initial muscle-damage bout on both cycling and running exercise. Therefore, the aim of Study 1 was to evaluate the effects of the same bout of muscle-damaging exercise on the physiological, metabolic and perceptual responses to sub-maximal running and cycling exercise.

Aims of Study 2 (Chapter 4)

Exercise-induced muscle damage is shown to increase oxidative metabolism at rest and during endurance exercise. However, it is not known whether oxygen uptake during recovery from endurance exercise is increased when experiencing symptoms of EIMD. Prolonged increases in oxygen uptake are evident hours after exercise (Gaesser & Brooks, 1984; Bahr, 1992). Coined the 'excess post-exercise oxygen consumption (EPOC)', the magnitude of EPOC is dependent upon the intensity of the exercise (Bahr & Sejersted, 1991; Bahr, 1992). Therefore, the observed increases in oxygen cost during rest and sub-maximal running when muscle is damaged could cause further increases in EPOC. Furthermore, endurance athletes often engage in repeated bouts of endurance exercise on the same day or in the days after, and, if $\dot{V}O_2$ is still elevated after exercising with muscle damage, reductions in exercise efficiency during subsequent endurance training sessions could occur (Bahr et al., 1991; Borsheim & Bahr, 2003). Therefore, the aims of Study 2 were to investigate the effects of EIMD on resting metabolic rate, sub-maximal endurance exercise and post-exercise oxygen consumption.

Aims of Study 3 (Chapter 5)

As described above, the RBE describes how an initial bout of muscle damaging exercise protects against a subsequent bout of the same exercise. While several studies have documented the RBE in the markers of EIMD, to date no study has examined whether the RBE can reduce the observed effects of EIMD on sub-maximal endurance exercise. Given that individuals take part in numerous resistance training sessions, rather than a single bout, it is pertinent to investigate if alterations to sub-maximal endurance performance are still evident after a repeated bout of

resistance exercise. Therefore, the aims of Study 3 were to investigate the effects of repeated bouts of muscle-damaging exercise on the physiological, metabolic, perceptual and kinematic responses during sub-maximal running exercise.

Aims of Study 4 (Chapter 6)

A low volume of EIMD protects the muscle against damage in the days after a high volume of muscle-damaging exercise (Howatson et al., 2007). However, whether the protective adaptation against a high volume of EIMD after a low volume of EIMD is transferable to endurance performance is yet to be elucidated. Such a study would be useful to endurance athletes considering periodized resistance training for the first time. For instance, performing a low volume of resistance exercise two weeks before engaging in concurrent training might precondition the muscle to withstand higher bouts of muscle-damaging exercise and its detrimental effects on endurance performance. Thus, the aims of Study 4 were to investigate whether a low volume of muscle-damaging exercise protects against a higher volume of muscle-damaging exercise and its negative impact on endurance exercise performance.

CHAPTER 2

REVIEW OF LITERATURE

2.1 The application of resistance training methods for improving endurance performance

Whilst traditional views advocate that aerobic training is the most effective method to enhance endurance performance (Temple, 1990), there are an increasing number of endurance athletes engaging in resistance training (Jung, 2003; Yamamoto et al., 2008). This is surprising given the distinctly contrasting adaptations to each modality when performed independently (Hoffman, 2002). However, given that endurance athletes are often required to sustain attacks, climb hills, or sprint in the final part of a competitive race (Tanaka & Swenson, 1998), the role of the neuromuscular system in producing muscular strength and power is arguably fundamental to success in endurance events (Noakes, 1988).

Several studies have found that maximal oxygen uptake ($\dot{V}O_{2\max}$) remains unchanged after resistance training, dispelling the view that such training impairs aerobic performance (Jung, 2003). Moreover, resistance training is reported to enhance running economy, which is viewed as a key determinant of distance running performance (see Saunders et al., 2004) and defined as the oxygen cost required for a given running speed (Jung, 2003). Storen et al. (2008) reported that eight weeks of heavy resistance training in trained distance runners significantly improved running economy by 5% without any change in $\dot{V}O_{2\max}$. Furthermore, three additional studies examining trained triathletes (Millet et al., 2002) and distance runners (Johnston et

al., 1997; Guglielmo et al., 2009) reported a 4 – 7% improvement in running economy after 4 – 14 weeks of heavy resistance training.

In all of these studies significant improvements in maximal strength were reported without concomitant changes in body mass, implying that neuromuscular adaptations were responsible for the initial strength gains as opposed to muscle hypertrophy (Sale, 1991). Moreover, neuromuscular characteristics, such as improved muscle coordination, increased motor unit activation and increased motor unit synchronization, are also attributed to the improved running economy after heavy resistance training. However, a direct measure of neuromuscular function (such as electromyography; EMG) has yet been employed to confirm this (Bonacci et al., 2009).

Neural adaptations are also known to be more prominent than muscle hypertrophy after explosive resistance training or plyometrics (Sale, 1991). Plyometrics improve the ability of the muscles to generate power through utilising the SSC (Turner et al., 2003). It has been suggested that plyometric training has the potential to enhance the 'stiffness' of the musculotendinous system, allowing the muscle to absorb and utilise elastic energy more effectively during SSC activities (such as distance running) (Spurrs et al., 2003). Indeed, it is postulated that adaptations from plyometric exercise enhance running economy by increasing the force generating capability of the muscle without relying on additional energy from oxidative or glycolytic sources (Saunders et al., 2004). Paavolainen et al. (1999) observed that nine weeks of explosive resistance training improved running economy by 8% and 5 km running performance by 3% in moderately trained cross-country runners. Improvements in indirect neuromuscular characteristics, such as 20 m sprint velocity, distance covered during five alternate forward jumps, and stance phase contact

times during fixed-intensity running, were all attributed to the improvements in endurance performance. Similarly, other studies have shown improvements in running economy and running performance after 6 – 9 weeks of plyometric training in moderately (Spurrs et al., 2003; Turner et al., 2003) and highly trained distance runners (Saunders et al., 2006). Improvements in running economy were linked to increased stiffness of the lower limb musculotendinous system, enhanced muscular power, and increased elastic energy return. (For a detailed review on the neuromuscular adaptations to resistance training and their implications, see Bonacci et al. (2009)) Contrary to previous evidence that has advocated the inclusion of resistance training in running-based endurance training programmes; the effects of resistance training on cycling endurance performance appear equivocal (Laursen et al., 2005). Whilst some studies report significant increases in maximal leg strength following heavy resistance training, this improvement was not met with concomitant enhancements in cycling endurance performance (Bishop et al., 1999; Jackson et al., 2007; Levin et al., 2009). In contrast, other studies have shown improvements in cycling performance after resistance training (Hickson et al., 1988; Bastiaans et al., 2001; Paton & Hopkins, 2005). However, the inclusion of high-intensity interval training in conjunction with resistance training (Paton & Hopkins, 2005) and the recruitment of inexperienced cyclists (Hickson et al., 1988) undermine the significance of these findings.

Finally, it appears that the majority of the literature strongly advocates for endurance athletes, particularly distance runners, to include resistance training in their normal training regime. However, while early strength gains are attributed to neuromuscular adaptation (without change in muscle size), long-term interventions might increase body mass and subsequently lessen endurance performance (Jung, 2003).

Furthermore, given the incidence of EIMD after unaccustomed and strenuous bouts of resistance training, the need to investigate the effects of muscle-damaging exercise on endurance performance is paramount for the athlete and coach (Byrne et al., 2004).

2.2 Proposed mechanisms of exercise-induced muscle damage

Despite a plethora of literature detailing the effects of muscle-damaging exercise on measures of athletic performance, the exact mechanisms responsible for EIMD are still the subject of debate (Pyne, 1994; Howatson & van Someren, 2008). Several authors have attempted to explain the series of physiological events associated with the initiation of skeletal muscle damage and the recovery process thereafter (Armstrong, 1984; 1986; 1990; Ebbeling & Clarkson, 1989; Armstrong et al., 1991; Pyne, 1994; Morgan & Allen, 1999; Warren et al., 2001; Kendall & Eston, 2002; Byrne et al., 2004), and it is the intention of this review to adopt the model of exercise-induced injury in skeletal muscle first suggested by Armstrong (1990). Armstrong's model proposes that exercise-induced muscle damage occurs in four key phases:

- (i) the *initial phase* – the event that triggers the injury process;
- (ii) the *autogenic phase* – when proteolytic systems begin to degrade cellular structures within the muscle fibre;
- (iii) the *phagocytic phase* – the inflammatory process responsible for the removal of cellular debris and the regeneration of damaged fibres;
- (iv) the *regenerative phase* – whereby the muscle fibre is repaired and restored to its normal state.

2.3 Initial events in muscle damage

The mechanism responsible for initiating muscle damage after eccentric exercise is thought to involve two possible pathways: metabolic and / or mechanical (Ebbeling & Clarkson, 1989; Armstrong, 1990; Armstrong et al., 1991; Pyne, 1994).

2.3.1 Metabolic pathway

The metabolic muscle damage paradigm hypothesises that the initial event of EIMD is caused by metabolic deficiencies within the exercising muscle (Tee et al., 2007). Metabolic events such as insufficient mitochondrial respiration, lowered pH, elevated intramuscular temperature and increased oxygen free radical production have all been attributed to the appearance of muscle damage (Armstrong, 1990; Kendall & Eston, 2002).

During exercise, mitochondrial respiration is increased to match the rate of adenosine triphosphate (ATP) synthesis to ATP hydrolysis (Armstrong et al., 1991; Pyne, 1994; Tee et al., 2007). However, during physical activity there is a point at which the demand for ATP within the muscle fibre exceeds ATP production (Krisanda et al., 1988). When this attenuation of ATP occurs in the vicinity of the calcium (Ca^{2+}) adenosine triphosphatase (ATPase) in the sarcoplasmic reticulum (SR) or sarcolemma, the removal of Ca^{2+} is compromised, causing an elevation in cytosolic Ca^{2+} , triggering muscle cell degradation (Armstrong, 1984; 1990; Armstrong et al., 1991; Tee et al., 2007). However, if insufficient mitochondrial respiration was primarily responsible for EIMD, then muscles which contract concentrically would show more damage than muscles which contract eccentrically (Ebbeling & Clarkson, 1989). This is because during equivalent force production

concentric actions exert a higher metabolic cost than eccentric actions (Bigland-Richie & Woods, 1976). However, that eccentric exercise requires less energy, yet yields a greater level of muscle damage, demonstrates that depleted ATP concentrations are unlikely to initiate EIMD (Armstrong, 1984).

A decrease in pH after muscle-damaging exercise is hypothesised to have a profound negative effect on the ability of the SR to uptake Ca^{2+} following muscular contraction (Kendall & Eston, 2002). Schwane et al. (1983) compared blood lactate concentrations and DOMS after level and downhill running, and reported significant elevations in blood lactate response during level running with no incidence of DOMS. Conversely, following downhill running participants reported increased DOMS despite no increase in blood lactate concentration. Thus, lowered pH is also unlikely to be the initiating metabolic mechanism responsible for muscle damage (Ebbeling & Clarkson, 1989; Armstrong, 1990; Armstrong et al., 1991).

Elevated intramuscular temperature resulting from EIMD could damage the structural integrity of the muscle, causing muscle fibre necrosis and breakdown of the connective tissue (Armstrong, 1984). Eccentric exercise is postulated to generate a higher local temperature than concentric actions (Armstrong, 1984; 1990). However, this hypothesis is not consistent across the literature (Abbot & Aubert, 1951; Nadel et al., 1972) and it is speculated that a reduced rate of heat removal rather than increased heat production within the muscle occurs after eccentric exercise (Armstrong et al., 1991).

Another mediator of metabolic muscle damage is the increased production of oxygen free radicals (Armstrong et al., 1991). Free radicals are molecules containing an unpaired electron in their outer valence shell, making them extremely exchangeable.

They have a strong oxidising effect, which is the basis for their destructive influence against the muscle cell (Kendall & Eston, 2002). Free radicals are thought to be produced through electron leakage within the mitochondria, membrane bound oxidases, and infiltrating phagocytic cells (Armstrong et al., 1991; Pyne, 1994; Kendall & Eston, 2002). After intense exercise, it is postulated that the generation of free radicals initiates membrane damage leading to muscle cell death (Armstrong, 1990; Pyne, 1994). However, whilst there is evidence that free radicals are produced as a result of EIMD (Ashton et al., 1999; Thompson et al., 2001), it is possible that free radicals are secondary to the initial muscle-damaging mechanism. Close et al. (2004) measured the amount of free radicals after 30 minutes of downhill running and observed that circulating markers of free radicals did not appear until 72 h after muscle-damaging exercise. Such observations indicate that the production of free radicals is not responsible for initiating muscle damage, but could be involved in mediating the recovery from EIMD (Close et al., 2004).

The metabolic muscle damage model proposes that the initial event of EIMD is caused by a disturbance in metabolic function. However, the contrasting argument behind each metabolic event implies that metabolic factors seem unlikely candidates for the initiation of EIMD (Howatson & van Someren, 2008).

2.3.2 Mechanical pathway

The mechanical hypothesis describes the initial event of EIMD as a direct consequence of mechanical loading on the muscle (Howatson & van Someren, 2008). Enoka (1996) explains that there is a significant reduction in the recruitment of motor units during eccentric activity, despite such muscle actions being able to generate more force compared to isometric or concentric contractions. This 'loading

profile' (high force with low muscle fibre recruitment) is postulated to place substantial mechanical stress on the muscle, and when repeated during an activity with a high eccentric component places sufficient stress on the muscle to induce failure and inevitable damage in some fibres (Byrne et al., 2004; Eston et al., 2004; Falvo & Bloomer, 2006; Tee et al., 2007).

The 'popping sarcomere' hypothesis explains that when the muscle is actively lengthened during eccentric activity, most of the length change is taken up by the weakest sarcomeres (Morgan, 1990). When this occurs on the descending limb of the length-tension curve, these sarcomeres progressively over-extend until they are beyond myofilament overlap (Morgan & Allen, 1999; Proske & Morgan, 2001). Upon relaxation of the muscle fibre, most of the over-extended sarcomeres re-interdigitate and are able to resume normal function. However, some sarcomeres fail to do so and remain over-extended (Morgan, 1990; Morgan & Allen, 1999; Proske & Morgan, 2001). Consequently, this leads to a rightward shift in the length-tension relationship, as a longer muscle length is required to achieve the same myofilament overlap observed prior to the eccentric activity (Proske & Morgan, 2001; Byrne et al., 2004; Eston et al., 2004).

Furthermore, during repeated eccentric exercise, broadening, smearing or total disruption occurs at the Z-line of the sarcomere. The over-extending sarcomere eventually disrupts, and it is proposed that as the Z-lines weaken the number of disrupted sarcomeres increases (Friden et al., 1983; Hortobagyi et al., 1998). Moreover, due to their non-uniform lengthening during eccentric contractions, once one or more sarcomeres become disrupted, the damage is thought to spread across to neighbouring functional sarcomeres, causing them to disrupt during subsequent contractions (Morgan & Allen, 1999; Morgan & Proske, 2004; Proske & Allen, 2005).

It is hypothesised that the extension of disrupted sarcomeres continues to increase, until a point at which membrane damage, including membranes of the SR, t-tubules, or the sarcolemma occurs (Morgan & Allen, 1999; Allen, 2001; Proske & Morgan, 2001; Proske & Allen, 2005). The uncontrolled movement of Ca^{2+} in the cytoplasm then ensues, triggering the autogenic stage of muscle damage (Morgan & Allen, 1999; Proske & Allen, 2005).

Whilst it is generally agreed that the initial phase of EIMD stems from the over-extension of sarcomeres, this theory is not universally accepted (Proske & Allen, 2005). An alternative view postulates that initial muscle damage is due to the failure to the excitation-contraction (E-C) coupling process (Warren et al., 2001). The E-C coupling process is a sequence of events that begins with the passage of the action potential along the plasmalemma and ends with the release of Ca^{2+} from the SR (Ingalls et al., 1998; Warren et al., 2001; Byrne et al., 2004; Eston et al., 2004). Evidence that the initiation of EIMD lies within the E-C coupling system has been shown through exposing injured mouse muscle to caffeine (Warren et al., 1993; Balnave & Allen, 1996). Caffeine promotes the release of Ca^{2+} from the SR, leading to the development of contracture within the muscle (Proske & Morgan, 2001; Warren et al., 2001). Thus, after muscle-damaging exercise, any reduction in caffeine-induced tension could be attributed to structural damage. However, caffeine-elicited force in mouse fibres is unchanged after EIMD, suggesting that alterations in Ca^{2+} release are accountable for most of the early tension deficit (Warren et al., 1993). Nevertheless, in amphibian muscle, the reduction in force as a result of EIMD is not recoverable through increasing Ca^{2+} release; therefore E-C

coupling dysfunction appears to be different between species (Morgan et al., 1996; Allen, 2001).

In humans, failure of the E-C coupling mechanism after EIMD has been shown through the force-frequency relationship (Byrne et al., 2004). The relationship, represented graphically as generated force (y-axis) against when a muscle is stimulated across a range of frequencies (x-axis), demonstrates exacerbated force losses at low frequencies (10 – 20 Hz) when compared to high frequencies (50 – 100 Hz) (Jones, 1996; Hill et al., 2001; Clarkson & Hubal, 2002; Byrne et al., 2004).

This phenomenon, termed low-frequency fatigue (LFF), is thought to be caused by reductions in SR Ca^{2+} release after EIMD (Edwards et al., 1977; Jones, 1996; Byrne et al., 2004; Nielsen et al., 2005; Dundon et al., 2008). However, increased muscular compliance as a result of over-extended sarcomeres can also reduce force at low frequencies (Allen, 2001). Therefore, LFF might be the direct result of structural damage to the myofibril and not necessarily evidence of E-C coupling failure (Jones, 1996; Allen, 2001; Clarkson & Hubal, 2002). Furthermore, if the initial cause for the early tension deficit after EIMD was due to E-C coupling failure, the shift in the length-tension curve could be interpreted as a reduction in activation, so that the muscle has to be stretched further in order to achieve maximal activation (Proske & Morgan, 2001). However, when tension is increased at long lengths (Katz, 1939; Morgan et al., 1996), reductions in activation or E-C coupling are unable to explain the change in the length-tension relation of the muscle (Morgan & Allen, 1999; Proske & Morgan, 2001). Therefore, it would appear more plausible to assume that E-C coupling failure is secondary to mechanical changes in the myofibril (Proske & Allen, 2005). Adopting the view of Proske and Morgan (2001), the initial events of

mechanical muscle damage would commence with the disruption of sarcomeres, leading to membrane damage and subsequent interference with the E-C coupling system.

2.4 Autogenic events during exercise-induced muscle damage

Regardless of the initiating event of EIMD (metabolic or mechanical), a common hypothesis that follows is the uncontrolled movement of Ca^{2+} into the cytoplasm, triggering the autogenic processes (Kendall & Eston, 2002). Calcium plays a vital role in the structure and function of the myofibril (Byrd, 1992). Following the movement of the action potential across the plasmalemma, Ca^{2+} release from the SR is necessary for the attachment of myosin to actin to produce muscular contracture. Furthermore, when released Ca^{2+} is re-accumulated into the SR by Ca^{2+} -ATPase muscular contraction is halted (Gissel, 2005).

Since extracellular Ca^{2+} concentration is considerably higher than that contained within the muscle cell, structural damage to the muscle membrane, after EIMD, could allow an influx of Ca^{2+} into the cell down its concentration gradient (Armstrong, 1984; 1986; 1990; Armstrong et al., 1991). When abnormally high concentrations of Ca^{2+} exist in the cytoplasm, several detrimental events occur to cause further damage to the muscle cell (Armstrong, 1984; 1986; Ebbeling & Clarkson, 1989; Howatson & van Someren, 2008). For example, high intracellular Ca^{2+} concentration activates Ca^{2+} -dependent proteolytic (calpains) and lipolytic (phospholipase A_2) pathways that result in the degradation of structural proteins within the muscle cell and subsequent cell death (Armstrong et al., 1991; Gissel & Clausen, 2001; Kendall & Eston, 2002; Gissel, 2005; Howatson & van Someren, 2008). The mitochondria are capable of accumulating large amounts of Ca^{2+} ; therefore in attempting to maintain cell

homeostasis, the mitochondria will sequester excess Ca^{2+} levels present in the muscle cell (Ebbeling & Clarkson, 1989; Gissel, 2005). However, if this storage capacity is exceeded, increased Ca^{2+} load will lead to the increased production of reactive oxygen species (ROS) and subsequent membrane damage (Gissel, 2005). Mitochondrial Ca^{2+} overload has also been suggested to impair cellular respiration and ATP production, which would have a detrimental effect on several cellular processes, including muscular contraction (Armstrong, 1984; 1986; Ebbeling & Clarkson, 1989; Gissel & Clausen, 2001; Gissel, 2005). Since Ca^{2+} is an initiating factor for muscular contraction, elevated intracellular Ca^{2+} could result in the uncontrolled contracture of a muscle fibre and may explain the increase in passive tension observed following muscle-damaging exercise (Armstrong, 1986; Morgan & Allen, 1999; Allen, 2001; Proske & Morgan, 2001; Proske & Allen, 2005).

2.5 The phagocytic phase of exercise-induced muscle damage

Exercise-induced muscle damage is associated with a well-documented inflammatory response that promotes the clearance of damaged tissue, eradicates microbial invaders, and initiates tissue repair (MacIntyre et al., 1995). It is postulated that this response is activated by the initial mechanical damage and is characterised by the infiltration of fluid and white blood cells (WBC) into the affected area (Pyne, 1994; Cannon & St. Pierre, 1998; Clarkson & Sayers, 1999; Clarkson & Hubal, 2002; Kendall & Eston, 2002; Butterfield et al., 2006). Tidball (1995) identified that at least two WBC populations respond to muscular injury; inflammatory cells responsible for the removal of cellular debris (neutrophils) and myogenic cells involved in the replacement of damaged tissue (macrophages).

Whilst it is still an area of much debate (see St. Pierre Schneider & Tiidus, 2007 for a review), it is generally believed that neutrophils are the first group of WBCs to infiltrate muscle tissue at the site of injury (Smith, 1991; MacIntyre et al., 1995; Cannon & St. Pierre, 1998; Peake et al., 2005; Butterfield et al., 2006). The exact mechanism responsible for neutrophil cell invasion is yet to be fully elucidated (Peake et al., 2005; Butterfield et al., 2006). The activation of calpain following muscle cell Ca^{2+} overload may be associated with neutrophil chemotaxis (Clarkson & Sayers, 1999; Gissel & Clausen, 2001; Kendall & Eston, 2002). Raj et al. (1998) found a relationship between Ca^{2+} -stimulated proteolysis and neutrophil accumulation, suggesting that calpains are involved in promoting neutrophil invasion (Clarkson & Sayers, 1999; Gissel & Clausen, 2001). Alternatively, elevated intracellular Ca^{2+} following EIMD could provide the signalling molecule responsible for the release of pro-inflammatory cytokines (Butterfield et al., 2006). Chin (2005) demonstrated that decreasing intracellular Ca^{2+} concentrations through blocking Ca^{2+} channels resulted in diminished cytokine production.

Cytokines are small polypeptides that act on the surface of target cells principally to alter cellular function (MacIntyre et al., 1995; Kendall & Eston, 2002; Butterfield et al., 2006). Following muscle-damaging exercise, myocytes release a number of cytokines, including interleukin (IL)- 1β and tumour necrosis factor- α (TNF α). These cytokines up-regulate endothelium-leukocyte adhesion molecules within the endothelial cells that line the walls of blood vessels. Activated endothelial cells then release additional IL- 1β , as well as IL-6 and IL-8, which have been demonstrated to attract neutrophils to the site of injury (Cannon & St Pierre, 1998; Kendall & Eston, 2002; Butterfield et al., 2006; Smith et al., 2008). Once infiltrated into the muscle tissue, the primary function of neutrophils is to attack and breakdown necrotic

myofibres and cellular debris. However, neutrophils can also produce high concentrations of cytolytic and cytotoxic molecules (including ROS) via a 'respiratory burst' that can aggravate existing damage from the mechanical insult. It is postulated that neutrophils are unable to distinguish between foreign and host antigens, therefore damage to the surrounding healthy tissue can also occur and may explain the so-called secondary inflammatory response (MacIntyre et al., 1995; Cannon & St. Pierre, 1998; Clarkson & Sayers, 1999; Clarkson & Hubal, 2002; Kendall & Eston, 2002; Tidall, 2005; Butterfield et al., 2006; St. Pierre Schneider & Tiidus, 2007; Smith et al., 2008).

After the accumulation of neutrophils, there is a subsequent infiltration of macrophages into the damaged tissue (Clarkson & Hubal, 2002). The mechanism responsible for the recruitment of macrophages remains unknown, however it is possible that damaged tissue releases similar signalling molecules to those used for recruiting neutrophils (Kendall & Eston, 2002; Butterfield et al., 2006). At present, there is much confusion within the literature regarding the function of macrophages once they invade the muscle tissue (Tidball, 2005; Butterfield et al., 2006). MacIntyre et al. (1995) postulated that macrophages, like neutrophils, are capable of producing cytotoxic enzymes and ROS leading to further tissue degradation. However in contrast, Arnold et al. (2007) demonstrated that macrophages once recruited by damaged tissue convert into anti-inflammatory phenotypes responsible for releasing growth factors. Therefore, it is possible that macrophages do not contribute to membrane disruption during the inflammatory response, but have a role in facilitating recovery (Butterfield et al., 2006; Smith et al., 2008).

Macrophages have also been shown to divide into two separate cell populations that perform contrasting functions at different phases of the inflammatory process. ED1⁺ macrophages infiltrate necrotic fibres within 24 h of neutrophil invasion and function as phagocytes to remove cellular debris from necrotic tissue. ED2⁺ macrophages are evident when muscle necrosis is complete and muscle regeneration commences. Therefore, these 'non-phagocytic' macrophages might serve as sources for growth factors and cytokines responsible for muscle cell proliferation (Tidball, 1995; Cannon & St. Pierre, 1998; Clarkson & Sayers, 1999; Butterfield et al., 2006; Smith et al., 2008). However, these findings have only been observed in animals and currently remain to be validated in human studies (Butterfield et al., 2006).

2.6 The regenerative phase of exercise-induced muscle damage

Muscle fibres are unable to perform cell division, thus during regeneration skeletal muscle requires the recruitment and activation of specialised precursor cells known as satellite cells. Satellite cells normally lie dormant outside muscle tissue, however upon stimulation after EIMD they migrate to the site of injury and commence proliferation (Cannon & St. Pierre, 1998). A crucial, but poorly understood stage, it is postulated that there is an associated division of satellite cells, which fuse into new established myotubes to form mature myofibrils (Jones & Round, 1990; Cannon & St. Pierre, 1998; Kendall & Eston, 2002). Furthermore, there is growing evidence that macrophage invasion is an essential pre-requisite for satellite cell stimulation. Macrophages, particularly ED2⁺, release a number of growth factors and cytokines that have been shown to promote satellite cell proliferation (Cannon & St. Pierre, 1998; Kendall & Eston, 2002; Butterfield et al., 2006; Smith et al., 2008).

2.7 Symptoms of exercise-induced muscle damage

The symptoms associated with EIMD are well documented and are used as markers to provide evidence of the magnitude and time course of muscle injury (Warren et al., 1999). Symptoms are exacerbated immediately after the bout of muscle damaging exercise and can last for several days, depending on the intensity and duration of the exercise adopted (Howatson & van Someren, 2008). Histological observation (light or electron microscopy) and changes in muscle function appear to be the most valid markers of EIMD (Paulsen et al., 2012). However, other symptoms such as the delayed onset muscle soreness (DOMS), swelling, muscle stiffness, decreases in range of motion and leakage of myofibre proteins into circulation (such as CK and myoglobin) have been used to provide indirect confirmation of EIMD (Kendall & Eston, 2002; Byrne et al., 2004; Eston et al., 2004).

Histological observations involve taking small biopsy samples (5 – 20 mg) from exercised and / or control muscle. The advantage of histological examinations is that they allow for the identification of abnormalities in the muscle, such as myofibrillar disruptions and the presence of inflammatory cells. Myofibrillar disruptions are evident immediately after exercise, can still be seen one week after EIMD, with the greatest disturbance found 1 – 4 days post-EIMD (Friden et al., 1983; Hikida et al., 1983; Newham et al., 1983; Hortobagyi et al., 1998; Raastad et al., 2010). However, despite histological evidence providing the ultimate sign of EIMD, questions are raised as to whether a small biopsy sample is representative of the whole muscle (Warren et al., 1999). Furthermore, there is also a risk of bias when taking repeated biopsy samples from the same muscle (Malm, 2001). Malm et al. (2000) observed no differences in inflammatory response in muscle samples taken from eccentrically-exercised and control muscle. This led them to suggest that the biopsy procedure

causes more damage and inflammation than muscle-damaging exercise. However, the findings from Malm et al. (2000) are undermined by the mild loss in muscle function (15% in the first 24 h post-EIMD), that had returned to normal within 48 h. It is possible that the eccentric exercise adopted did not result in appreciable damage to induce fibre necrosis and the ensuing inflammatory response. Prior and subsequent research that used greater intensity muscle-damaging exercise have shown evidence of myofibril disruption and fibre necrosis (Jones et al., 1986; Child et al., 1999; Beaton et al., 2002; Lauritzen et al., 2009; Paulsen et al., 2010). It should be noted that the cost, the expertise required and the discomfort experienced by participants may lead investigators to examine more indirect markers of EIMD. The most popular indirect markers of EIMD amongst human research are DOMS, CK activity and muscle function (Warren et al., 1999).

2.7.1 Delayed onset muscle soreness

Delayed onset muscle soreness is the most frequently reported marker of EIMD (Warren et al., 1999) and is classified by sensations of pain, tenderness, and stiffness upon palpation or movement of the damaged muscle (Armstrong, 1984; Ebbeling & Clarkson, 1989; Jones & Round, 1990; Miles & Clarkson, 1994; Cheung et al., 2003). Delayed onset muscle soreness normally appears between 8 and 24 h after cessation of muscle-damaging exercise, and peaks 24 – 48 h later. All discomfort eventually subsides within 96 h and participants are usually pain-free after 5 to 10 days, depending on the intensity and/or duration of the muscle-damaging exercise performed (Jones et al., 1986; Ebbeling & Clarkson, 1989; Clarkson et al., 1992; Cleak & Eston, 1992a).

Whilst DOMS is an appropriate term to describe the time course of the sensations of pain, tenderness and stiffness, it conveys no information about aetiology (Byrne et al., 2004). The sensation of pain in skeletal muscle is believed to be signalled by myelinated group III and unmyelinated group IV afferent fibres located in the musculotendinous junctions and fascial sheaths (Armstrong, 1984; Ebbeling & Clarkson, 1989; Jones & Round, 1990; Cleak & Eston, 1992a; Kendall & Eston, 2002). It is hypothesised that the stimulation of group III fibres results in the transmission of a brief, sharp pain whereas the activation of group IV fibres yields a duller, longer-term pain response that is more indicative of DOMS (Ebbeling & Clarkson, 1989; Jones & Round, 1990; Kendall & Eston, 2002).

Several theories have been postulated to explain the underlying mechanisms of the sensitisation of pain receptors after EIMD. For example, the accumulation of noxious chemicals (such as prostaglandin, bradykinin, and histamine), increased muscle temperature, and elevated pressure from tissue oedema have all been implicated in activating afferent fibres to produce DOMS. To date, the initiating stimulus responsible for DOMS has still to be elucidated. However, whilst it appears that no single theory can explain muscle soreness, it has been suggested that the onset of DOMS might occur due to a complex interaction of numerous events. For a more detailed account of the possible causes of DOMS, the reader is referred to the reviews by Armstrong (1984), Cleak and Eston (1992a), MacIntyre et al. (1995) and Cheung et al. (2003).

Numerous methods have been adopted to quantify the magnitude of DOMS following EIMD. Subjective measures of muscle soreness are obtained from visual analogue scales where a numerical value is selected to represent a corresponding adjective. Objective measures of muscle soreness have also been established and

are gained by recording the amount of force required to elicit a painful response (Jones & Round, 1990; Byrne et al., 2004). However, such measurements have been criticised due to the one-dimensional approach quantifying muscle soreness. Pain is multi-faceted, comprising a sensory dimension that includes temporal, spatial and pressure aspects; an affective dimension that incorporates emotional responses to pain; and an evaluative dimension that encompasses cognitive facets of pain (MacIntyre et al., 1995; Cleather & Guthrie, 2007). The McGill Pain Questionnaire, which incorporates numerous aspects of pain, is suggested to provide a more appropriate assessment of DOMS. However, in a study by Cleather and Guthrie (2007), which measured DOMS following eccentric exercise using both a one-dimensional measurement of pain and a more complex multi-dimensional tool, it was observed that the time course of DOMS was the same for each method.

Despite the popularity of DOMS amongst the battery of tests to measure EIMD, it should not be used as an indicator of functional impairment or the magnitude of muscle damage (Byrne et al., 2004). After EIMD, muscle function has been shown to be impaired before muscle soreness arises. Moreover, decrements in muscle function are still evident after muscle soreness has dissipated (Jones et al., 1986; Warren et al., 1999; Nosaka et al., 2002a; Byrne et al., 2004). Muscle soreness responses to a bout of muscle-damaging exercise vary greatly between individuals. For example, participants might rate muscle soreness at 40 cm on a 100 cm VAS even though their perceived level of pain is medium, whilst, others might rate soreness at 10 cm despite their level of pain being severe. Due to its subjective and individual nature, questions are raised as to whether it is possible to compare perceptions of DOMS amongst participants (Nosaka et al., 2002a). Jones and Round (1990) also documented that perceptions of noxious stimuli differ between

individuals and vary depending upon a person's mood, health and hormonal status. Melzack (1982) suggested that pain is not a measure of the amount of bodily damage and can be influenced by attention, anxiety, suggestions and other psychological determinants. For example, previous memories of DOMS might influence future sensations of muscle soreness following EIMD.

2.7.2 Effects of exercise-induced muscle damage on myofibre proteins

The increased appearance of myofibre proteins in the blood is widely used to provide indirect evidence of muscle damage (Armstrong, 1986; Clarkson & Ebbeling, 1988; Hortobagyi & Denahan, 1989; Clarkson & Hubal, 2002; Brancaccio et al., 2007). In particular, CK, lactate dehydrogenase, aspartate aminotransferase, and myoglobin have been measured frequently as indicators of muscle damage (Pyne, 1994; Sorichter et al., 1999; Warren et al., 1999; Clarkson & Hubal, 2002). Although all these proteins have been shown to be elevated following EIMD, the measurement of CK is the most prevalent within the literature (Clarkson & Hubal, 2002).

Creatine kinase is an intramuscular enzyme responsible for maintaining adequate ATP levels during muscular contraction by catalysing the reversible exchange of high-energy phosphate bonds between phosphocreatine and adenosine diphosphate (Hortobagyi & Denahan, 1989; Friden & Lieber, 2001; Brancaccio et al., 2007). Following muscle-damaging exercise, the release of CK from the muscle tissue into the circulating blood stream is interpreted as evidence of breakdown or increased permeability of the muscle cell membrane (Armstrong, 1986; Hortobagyi & Denahan, 1989; Friden & Lieber, 2001). The time course of this release appears to be dependent on the type of muscle-damaging exercise adopted. For example, following ecologically valid modes of EIMD, such as resistance training, plyometrics,

downhill running and intermittent team sports, CK values peak after approximately 24 h (Byrne & Eston, 2002a; Twist & Eston, 2005; Chen et al., 2007; Magalhaes et al., 2010; McLellan et al., 2010). In contrast, studies that have adopted high force eccentric contractions using isokinetic dynamometry have shown CK to increase gradually, with peak activity occurring 4 – 7 days post-exercise (Clarkson et al., 1992; Nosaka & Clarkson 1992; 1997; Byrne et al., 2001). The mechanism behind this exercise-dependent CK response appears to be unknown. Jones et al. (1986) did postulate that the intensity of the muscle-damaging exercise might determine the rate of muscle breakdown and subsequent CK release, though this has remained unconfirmed.

Changes in the blood levels of CK following EIMD are often assumed to reflect the magnitude of muscle damage (Friden & Lieber, 2001). However, Warren et al. (1999) undermine the use of myofibre proteins as a marker of muscle damage by suggesting that alterations in CK are not concomitant with decreases in muscle function following EIMD. This is supported by research that has shown a poor temporal relationship between serum CK leakage and the force-generating capacity of skeletal muscle after EIMD (Margaritis et al., 1999; Friden & Lieber, 2001).

Several studies have also demonstrated that CK activity shows a large inter-participant variability. For example, peak CK values have been reported to range from 96 – 34,500 U·l⁻¹ after a bout of muscle-damaging exercise (Newham et al., 1983; Clarkson et al., 1992; Nosaka & Clarkson, 1996). Various factors have been hypothesised to explain this phenomenon, including sex, race, genetics, muscle mass, muscle fibre distribution and the training status of recruited participants (Nosaka & Clarkson, 1992; Vincent & Vincent, 1997; Clarkson & Hubal, 2002; Clarkson et al., 2005; Brancaccio et al., 2007; Magal et al., 2010). However, there is

still no clear explanation for the large variability in CK after a single bout of EIMD (Ebbeling & Clarkson, 1989; Clarkson & Hubal, 2002; Chen, 2006).

Whilst CK provides an indirect marker of disruption to the muscle cell membrane, it lacks muscle fibre-type specificity and is unable to confirm disruption to the myofibril after EIMD (Sorichter et al., 1999). Alternatively, studies have examined plasma levels of myosin heavy chains (MHC) (Mair et al., 1992) and skeletal troponin I (sTnI) (Sorichter et al., 1997) after EIMD. Myosin heavy chains and sTnI are structurally bound contractile proteins of the thick and thin filaments within the sarcomere (Sorichter et al., 2001), thus, their appearance in plasma after EIMD confirms structural breakdown of the muscle. Mair et al. (1992) observed that MHC were evident in circulation within 1 – 3 days and were still detectable until 10 days after EIMD induced from downhill running. Similarly, Sorichter et al. (1997) reported that plasma concentrations of MHC were evident within 24 h, peaked at 48 h and were still increased 9 days after EIMD. In contrast, increases in plasma sTnI were found to peak at 6 h and remained elevated for 1 – 2 days after EIMD. This led Sorichter et al. (1997) to suggest that sTnI is an early marker of EIMD and is able to reflect the rapid disruption to the myofibril after EIMD. It is suggested that sTnI is more susceptible to calpain digestion than MHC, which might explain its earlier release from the muscle after EIMD (Sorichter et al., 1999).

2.7.3 Effects of exercise-induced muscle damage on muscle function

The most effective indirect method of determining the magnitude and time course of EIMD is the measurement of the force generating capacity of skeletal muscle (Warren et al., 1999; Byrne et al., 2004). Reductions in muscle function, which relate to reductions in muscle strength and power, occur immediately after EIMD and

continue to be evident whilst other markers, such as perceived muscle soreness, subside (Byrne et al., 2004). Measuring isometric strength appears to be the most widely used method to assess muscle function after muscle-damaging exercise (Clarkson et al., 1992; Howell et al., 1993; Warren et al., 1999; Byrne et al., 2001; Sayers & Clarkson, 2001; Byrne & Eston, 2002b; Byrne et al., 2004). The method requires subjects to perform maximal voluntary contractions (MVC) on the damaged muscle group against an immovable object without a change in joint angle (Abernethy et al., 1995). When muscle damage is present, isometric strength will reduce immediately and research has shown that recovery (of strength) is generally slow and prolonged. For example, elbow flexor isometric strength has been reported to decrease by 50 – 60% in the hours immediately following EIMD, whilst recovery periods have taken up to two weeks (Cleak & Eston, 1992b; Sayers & Clarkson, 2001; Byrne et al., 2004). Isometric strength within the knee and ankle extensors has also been shown to incur immediate and prolonged reductions; though the loss is not as extensive (~35% loss) as the elbow flexors and recovery is quicker (4 – 7 days) (Byrne & Eston 2002a; 2002b; Eston et al., 2004). This is attributed to the relative inactivity of the elbow flexors in comparison to the knee and ankle extensors (Byrne et al., 2004; Eston et al., 2004).

Several studies have demonstrated that the optimal angle for isometric torque generation is shifted to the right after muscle-damaging exercise (Jones et al., 1997; Whitehead et al., 1998; Brockett et al., 2001; Byrne et al., 2001; McHugh & Tetro, 2003; Bowers et al., 2004; Chen et al., 2007a). Jones et al. (1997) reported a 3.9° shift in optimal angle for torque production after downhill running; however optimal angles returned to baseline values by 2 days post-EIMD. Rightward shifts in optimal angle of 3.4 – 15.6° have been shown in the quadriceps for up to 8 days after

stepping exercise (Bowers et al., 2004) and a 4° shift was still evident in the elbow flexors 2 – 3 weeks after eccentric exercise (Chen et al., 2007a). Discrepancies between studies might be due to different modes of muscle-damaging exercise; however, they do provide evidence that the length-tension relationship is shifted to the right after EIMD. Consequently, a longer muscle is required to achieve the same myofilament overlap and maximal torque as a result of overextended sarcomeres following eccentric exercise (Proske & Morgan, 2001; Byrne et al., 2004; Eston et al., 2004).

Due to the fixed nature of assessing isometric strength, the method fails to resemble the dynamic nature of most sporting tasks (Abernethy et al., 1995; Baltzopoulos & Gleeson, 2009). Alternatively, isokinetic dynamometry has also been adopted to assess dynamic muscle function after muscle-damaging exercise. However, the research findings appear equivocal, particularly with respect to the losses in muscle strength at differing contraction speeds. Several studies have observed that strength loss is greater at slow angular velocities than at faster contractions (Gibala et al., 1995; Deschenes et al., 2000; Michaut et al., 2002; Twist & Eston, 2007; Twist et al., 2008; Highton et al., 2009). Michaut et al. (2002) reported that isokinetic concentric strength in the elbow flexors was reduced to lesser extent at 4.19 rad·s⁻¹ than at 1.05 rad·s⁻¹ after EIMD. Similar findings were observed by Deschenes et al. (2000), who found that isokinetic concentric strength in the knee extensors was not significantly reduced at 3.14 rad·s⁻¹; however, strength at 1.09 rad·s⁻¹ was reduced for 48 h post-EIMD. Conversely, studies have found strength to recover more slowly after high velocities of movement than slow velocities (Friden et al., 1983; Golden & Dudley, 1992; Eston et al., 1996). Golden and Dudley (1992) demonstrated that concentric strength at 3.14 rad·s⁻¹ was slower to return to pre-damage values than strength loss

at $1.05 \text{ rad}\cdot\text{s}^{-1}$. Likewise, Eston et al. (1996) observed that strength loss at $0.52 \text{ rad}\cdot\text{s}^{-1}$ returned to pre-EIMD values within 4 days, while losses at $2.79 \text{ rad}\cdot\text{s}^{-1}$ took 7 days to return to baseline. In contrast to both of these responses, Byrne et al. (2001) reported no velocity-dependent losses in isokinetic muscle force following eccentric exercise.

Studies that show losses in isokinetic strength are greatest at higher angles of velocity support the theory that type II fibres are predominately damaged as a result of eccentric exercise. Observations that strength decrements are less at higher angular velocities than lower velocities contradict this view (Byrne et al., 2004). It is posited that slower movement velocities require a higher effort to produce force (Deschenes et al., 2000; Michaut et al., 2002); therefore neural inhibition after EIMD might have reduced force at slower velocities to protect the muscle against further damage (Westing et al., 1991). However, it should be noted that comparisons between contrasting studies are made difficult by different protocols used to induce muscle damage (Byrne et al., 2004).

2.7.3.1 Sensory perception of force, limb position and joint reaction angle after exercise-induced muscle damage

In addition to decrements in isometric and isokinetic muscle function, several studies have shown that EIMD disturbs proprioception (namely sense of force production and limb position sense) (Saxton et al., 1995; Brockett et al., 1997; Carson et al., 2002; Proske et al., 2003; 2004; Weerakkody et al., 2003; Walsh et al., 2004; 2006; Allen et al., 2007; Paschalis et al., 2007a; 2008a). Saxton et al. (1995) observed that participants' ability to match force produced in the elbow flexors was prone to large errors as a result of EIMD. In a force-matching task, the elbow flexors of one arm

(the reference arm) generated a specified level of isometric force using visual feedback. Participants were then instructed to match the same level of force using their other arm (the indicator arm) without any visual aid. After inducing muscle damage in the indicator arm, participants were found to significantly undershoot the target force (35% isometric MVC) generated in the undamaged arm. Moreover, when the muscle-damaged arm acted as the reference arm, force-matching errors occur in the opposite direction. For example, when the damaged arm was required to sustain 30% of isometric MVC it was met with significantly higher matching torques in the undamaged arm (Weerakkody et al., 2003; Proske et al., 2003; 2004).

Brockett et al. (1997) speculated that damage to the Golgi tendon organ (GTO) as a result of eccentric exercise was responsible for the altered sense of force. Gregory et al. (2002) tested this hypothesis in cat gastrocnemius muscle and found that despite the loss of muscle function, the ability of the GTO to signal changes in muscle tension was not affected by EIMD. They concluded that the alteration in sense of force after EIMD was due to the centrally derived sense of effort, with any peripheral contribution likely to be small.

The sense of effort is thought to be informed by a perceived central motor command that is directed to the muscle (Carson et al., 2002; Gregory et al., 2002; Proske et al., 2004). However, Carson et al. (2002) and Weerakkody et al. (2003) reported that despite participants estimating the force applied by both the damaged and undamaged arm to be the same, the motor command was consistently higher in the damaged arm. This implied that the sense of effort was not based upon a corollary discharge of the central motor command. Instead, Carson et al. (2002) proposed that the relationship between the sense of effort and the central motor command is altered as a result of EIMD, and suggested that the sense of effort is associated with

activity in the neural centres upstream of the motor cortex. Proske et al. (2003) argued that the central motor command undergoes a complex series of excitability changes after exercise, and whilst it appears that motor command is increased immediately after muscle-damaging exercise it subsides after 24 h. They suggested that disturbances in sense of force were due to muscle soreness reducing motor cortical excitability, which reduced motor output to protect the muscle from further damage.

In addition to the changes in sense of force, it has also been shown that positional limb sense is disturbed after EIMD (Saxton et al., 1995; Brockett et al., 1997; Walsh et al., 2004; 2006; Allen et al., 2007; Paschalis et al., 2007a; 2008a). In a limb position-matching study, whereby a reference arm was placed at a pre-determined angle and participants were asked to match the angle with the opposite arm, Saxton et al., (1995) reported that a muscle-damaged forearm adopted a more flexed position when attempting to match an undamaged reference arm. Brockett et al. (1997) observed the opposite, with the damaged arm adopting a more extended position after EIMD. However, subsequent findings from Walsh et al. (2004) reaffirm those of Saxton et al (1995) that the muscle-damaged arm matched the undamaged arm with a more flexed position. The discrepancy between the studies were attributed to Brockett et al. (1997) using a milder bout of muscle-damaging exercise (Gregory et al., 2004). Paschalis et al. (2007a; 2008a) examined positional sense in the lower limb and observed that both the knee extensors and knee flexors adopted a more extended position after EIMD. Despite the contrasting findings between upper and lower limbs, position sense is a necessity for human movement. Therefore, any alteration to limb position sense as a result of EIMD could have

implications for future exercise and heighten the risk of further injury (Paschalis et al., 2007a).

The sense of position is thought to be provided by signals from the muscle spindles (McCloskey, 1978; Gandevia, 1996). Brockett et al. (1997) proposed that damage to the muscle fibres after EIMD could also damage the intrafusal fibres of the muscle spindles, leading to disturbances in muscle spindle function and errors in positional sense. It was suggested that an increase in passive tension after EIMD could mechanically unload the muscle spindles, which could lower their passive discharge rates, enabling greater muscle extension (Paschalis et al., 2007a). However, the responsiveness of the muscle spindles in anaesthetised cats was not altered after EIMD, which implies that changes in limb-position sense following EIMD are not due to disturbances to the muscle spindles (Gregory et al., 2004). Alternatively, Paschalis et al. (2008a) postulated limb position sense is changed to reduce the pressure on cutaneous receptors as a result of the inflammatory response associated with EIMD. Moreover, Walsh et al. (2004) observed that the errors in matching limb-position between the damaged and undamaged arm correlated with the drop in force. They suggested that the damaged limb required more effort to maintain a position against the force of gravity. Therefore, in order to reduce the effort, the damaged limb adopted a more flexed position, whereby less force was needed to maintain that position against gravity (Walsh et al., 2004; Gregory et al., 2004).

Alongside alterations to limb position sense, Paschalis et al. (2007a; 2008b) reported disturbances to knee joint reaction angle following EIMD. To ascertain the reaction angle, participants were required to stop the fall of their limb as soon as it was released from a pre-determined angle. The corresponding angle from which the limb

moved before participants managed to halt the fall was accepted as the knee joint reaction angle (Paschalis et al., 2007a; 2008b). Reaction angle in both the knee extensors and flexors was increased for up to 3 days after muscle-damaging exercise. Paschalis et al. (2007a; 2008a) posited that sarcomere disruption as a result of EIMD prevented the muscle from achieving the same pre-damage tension required to halt the fall of the limb. Alternatively, the altered knee joint reaction angle was attributed to an increase in knee extensor and flexor compliance following eccentric exercise. If compliance had increased after EIMD, the knee extensors and flexors would have been stretched further during the release test before the muscle spindles were able to signal the fall of the limb (Paschalis et al., 2007a; 2008a). That, EIMD is shown to alter reaction time, individuals should be considerate of the consequences of exercise requiring quick reactions in the days following EIMD.

2.7.3.2 Effects of exercise-induced muscle damage on neural control

Using EMG to examine motor unit activity, several studies have shown that neuromuscular control during sub-maximal contractions is disturbed as a result of EIMD (Komi & Viitasalo, 1977; Newham et al., 1983; Nicol et al., 1991; Carson et al., 2002; Weerakkody et al., 2003; Semmler et al., 2007; Dartnall et al., 2008; Turner et al., 2008; Plattner et al., 2011). Komi and Viitasalo (1977) reported that EMG activity in the eccentrically exercised knee extensors was elevated for up to 48 h after exercise, whilst in the control concentrically-exercised knee extensors recovery was complete within 48 h. They suggested that the muscle soreness associated with the eccentric exercise was responsible for the increase in central activation. However, studies have observed that EMG activity returns to baseline when muscle soreness increases (Berry et al., 1990; Hamlin & Quigley, 2001; Carson et al., 2002;

Weerakkody et al., 2003), which suggests that muscle soreness is not responsible for an elevation in EMG activity, but might contribute to a down-regulation in EMG after EIMD to protect the muscle against further damage (Proske et al., 2003). Peak EMG activity during maximal contractions is shown to decrease after EIMD and is also attributed to a decline in neural drive to protect the muscle (Komi & Rusko, 1974; Nicol et al., 1991; Plattner et al., 2011; Zhou et al., 2011).

There are numerous postulated explanations for the increase in sub-maximal EMG activity post-EIMD. The most popular hypothesis contends that when the muscle is damaged or fatigued, motor unit recruitment is increased in order for the muscle to achieve the same required force as before EIMD (Weerakkody et al., 2003; Proske et al., 2003; Semmler et al., 2007; Dartnall et al., 2008; Turner et al., 2008; Plattner et al., 2011). Nevertheless, a reduction in force after fatiguing concentric exercise would also be expected to increase motor unit recruitment during subsequent sub-maximal contractions; however, no change in EMG activity has been observed (Weerakkody et al., 2003; Semmler et al., 2007). Alternatively, Turner et al., (2008) observed an increase in antagonist muscle activity after EIMD. They speculated that this was a motor strategy to increase joint stability to maintain movement as a result of muscle weakness following EIMD. Dartnall et al. (2008) reported evidence of an increase in motor unit synchronisation, which might have contributed to the increased EMG activity during sub-maximal isometric contractions after EIMD. The alteration in the length-tension relationship has also been posited to explain the disturbance in EMG (Weerakkody et al., 2003; Turner et al., 2008). After eccentric exercise, the remaining functioning sarcomeres adopt a shorter length to compensate for the overstretched sarcomeres. At shorter lengths a higher level of activation is required to achieve a given level of force (Turner et al., 2008).

Therefore, the increase in EMG at sub-maximal contractions could be the result of individuals trying to attain a given force, but operating at a shorter muscle length (Weerakkody et al., 2003; Turner et al., 2008). It is probable that the disturbance in sub-maximal EMG is due to a combination of all factors mentioned. Whilst, research has shown that sub-maximal EMG activity is increased during isometric and isokinetic strength post-EIMD, it remains to be seen if EIMD affects neuromuscular control during endurance exercise.

2.7.3.3 Effects of exercise-induced muscle damage on electromechanical delay

In a recent study, Howatson (2010) reported that the negative effects of EIMD on measures of muscle function were extended to electromechanical delay (EMD). Electromechanical delay is the time lag between the onset of muscle activation and force production (Cavanagh & Komi, 1979). It was found that after muscle-damaging exercise, comprising 45 maximal eccentric contractions of the elbow flexors, EMD during isometric and isokinetic contractions was increased for up to 96 h. Howatson (2010) suggested that the increase in EMD was due to the loss of cell membrane and sarcolemma integrity following EIMD, which subsequently could impact action potential propagation, E-C coupling and force transmission along the series elastic component. Interestingly, alterations to EMD after EIMD were still evident when MVC showed signs of full recovery. This led Howatson (2010) to suggest that despite MVC returning to pre-EIMD levels, caution should still be instilled when prescribing exercise in the days after muscle damage, particularly if the activity requires a combination of high force with a high degree of motor control.

2.7.4 Individual susceptibility to exercise-induced muscle damage

Whilst, the symptoms associated with EIMD are well documented, the extent of muscle damage amongst individuals is notably variable (Sayers et al., 1999; Sayers & Clarkson, 2001; Sewright et al., 2008; Hubal et al., 2010). For example, Hubal et al. (2010) observed that muscle soreness 12 h after 50 maximal eccentric contractions in the elbow flexors ranged from 0 to 94 mm on a 100 mm VAS. Likewise, MVC strength immediately after the muscle-damaging exercise ranged from +29% to -91% and was still highly variable 7 days later (ranging from +36% to -86%). Creatine kinase activity was found to be the most variable symptom, with values 4 days post-EIMD ranging from 85 to 80550 U·l⁻¹. Why some individuals have a greater pre-disposition to EIMD than others is still not fully understood. However, several potential mechanisms have been suggested, including sexual dimorphism, statin use, nutritional supplement use and pre-existing muscle disease (Hyldahl & Hubal, 2013). There is also increasing evidence that genetics are responsible for inter-individual responses to EIMD (Clarkson et al., 2005; Devaney et al., 2007; Yamin et al., 2007; Hubal et al., 2010). Indeed, studies have recently identified a number of genetic polymorphisms that are associated with muscle damage variability. Clarkson et al. (2005) observed that rare alleles of myosin light chain kinase (MLCK; a gene involved in muscle contraction) are associated with increases in CK and strength loss after EIMD. Likewise, Devaney et al. (2007) found that rare alleles of insulin-like growth factor II (IGF2; a gene contributing to muscle growth) are associated with increases in muscle soreness, CK activity and strength loss after muscle-damaging exercise. Several other genes have also been shown to harbour polymorphisms that can increase an individual's susceptibility to EIMD, such as alpha-actinin 3 (ACTN3; Clarkson et al., 2005), angiotensin converting enzyme

(ACE; Yamin et al., 2007), IL-6 (Yamin et al., 2008), chemokine ligand 2 (CCL2; Hubal et al., 2010) and creatine kinase-muscle isoform (CKMM; Heled et al., 2007).

2.8 Adaptation and the repeated bout effect

A morphological adaptation after an initial bout of EIMD demonstrates that when eccentric exercise is repeated, myofibrils are more resistant to future bouts of muscle-damaging exercise (Friden & Lieber, 2001). Indeed, it has been shown that after recovery from an episode of eccentric-biased exercise, performance of a repeated bout of the same muscle-damaging exercise yields muscle damage of a lower magnitude (McHugh et al., 1999a; McHugh, 2003; Howatson & van Someren, 2008). Referred to as the 'repeated bout effect' (RBE), this protective adaptation is characterised by a reduced deficit in muscle function, a lowered perception of muscle soreness, a reduction in the appearance of intramuscular proteins in blood circulation and an attenuated inflammatory response (Byrnes et al., 1985; Newham et al., 1987; Nosaka & Clarkson, 1995; Pizza et al., 1996; Hortobagyi et al., 1998; Howatson et al., 2007; Smith et al., 2007). The RBE can occur within 2 – 3 days of a single episode of EIMD (Paddon-Jones et al., 2000; Nosaka & Newton, 2002; Chen, 2003; Lavender & Nosaka, 2008) and last for up to 6 months (Nosaka et al., 2001a). Furthermore, the muscle-damaging exercise does not have to result in symptoms of EIMD in order to infer a protective effect (Clarkson & Tremblay, 1988; Brown et al., 1997; McHugh, 2003). Nosaka et al. (2001b) demonstrated that two maximal eccentric contractions were sufficient to protect the elbow flexors against 24 maximal contractions performed two weeks later. Likewise, Chen et al. (2007a) confirmed that sub-maximal eccentric exercise (30 repetitions at 40% MVC) protected against

muscle damage after maximal eccentric exercise (30 repetitions at 100% MVC) performed two weeks later.

The underlying mechanism responsible for the RBE remains unknown; however several studies posit that the protective adaptation is due to neural and peripheral mechanisms, that work in combination or independently of one another (McHugh, 2003; McHugh et al., 1999a). The neural theory proposes that the RBE is due to an alteration in motor unit recruitment. Golden and Dudley (1992) suggested that the muscle is resistant to a second bout of EIMD due to a more efficient recruitment of motor units. Using surface EMG to monitor muscle recruitment, Warren et al. (2000) and Chen (2003) demonstrated a 20-30% reduction in the frequency content (MF) of the EMG signal during the second of two bouts of EIMD. However, both studies did not allow for a full recovery before the repeated bout of EIMD was performed, speculating that the changes in MF may have been due to muscle damage still evident from the first bout (Howatson et al., 2007). In attempts to address this limitation, Howatson et al. (2007) ensured symptoms of muscle damage had returned to baseline before the second bout of EIMD, and still found MF was decreased by 10% during Bout 2. More recently, Starbuck and Eston (2012) reported a 31% reduction in MF in the elbow flexors during the second of two bouts of eccentric exercise, separated by two weeks. It is believed that a reduction in MF indicates a higher reliance on slow twitch muscle fibres (Warren et al., 2000). Given that slow twitch fibres are more resistant to muscle damage (Friden et al., 1983), it is plausible that a greater recruitment decreases the stress on susceptible fast twitch fibres and protects the muscle against a repeated bout of EIMD (McHugh et al., 2001). Alternatively, a decrease in MF could be attributed to an increase in motor unit synchronisation (Pierrynowski et al., 1987). Motor unit synchronisation is the

tendency for two or more motor units to fire simultaneously during muscular contraction. Therefore, an increase in synchronisation during a repeated bout of EIMD might provide a greater distribution of workload among active muscle fibres (Pierrynowski et al., 1987; Nosaka & Clarkson, 1995; McHugh et al., 2001).

Numerous studies have observed that EMG amplitude is greatly increased after eccentric training when compared against concentric training (Komi & Buskirk, 1972; Hortobagyi et al., 1996a; 1996b). Hortobagyi et al. (1996b) reported that 12 weeks of eccentric strength training in the knee extensors resulted in a 188% increase in EMG activity, compared with a 28% increase in EMG activity after 12 weeks of concentric strength training. An increase in EMG amplitude during a repeated bout of EIMD would also reflect a redistribution of contractile stresses over a greater number of muscle fibres (McHugh et al., 1999a; McHugh, 2003). However, several studies have demonstrated that EMG amplitude is unchanged after repeated bouts of muscle-damaging exercise (Warren et al., 2000; McHugh et al., 2001; Chen, 2003; Howatson et al., 2007; Starbuck & Eston, 2012), suggesting that an increase in motor unit recruitment during a second bout of EIMD does not contribute to the attenuation of muscle damage.

Whilst the aforementioned studies confer evidence of a neural adaptation, research has shown that the RBE can occur independently of a neural mechanism (McHugh et al., 1999a; McHugh, 2003). Nosaka et al. (2002b) demonstrated that a single bout of electrically stimulated eccentric contractions attenuated the symptoms of muscle damage after a repeated bout of the same stimulation protocol. Since electrical stimulation bypasses the involvement of the central nervous system (CNS), the authors concluded that the mechanism responsible for the RBE is peripherally

mediated, with the involvement of the CNS being minimal. The findings of Black and McCully (2008) and Kamandulis et al. (2010) lend further support to the theory that the RBE is related to structural changes within the muscle and not changes in muscle recruitment. Furthermore, McHugh et al. (2001) were unable to find evidence of a neural adaptation after repeated bouts of sub-maximal eccentric exercise. Despite these findings, a neural mechanism responsible for the RBE cannot be dismissed, particularly during maximal eccentric exercise that does not require electrical stimulation.

The peripheral theory contends that the RBE is due to mechanical and/or cellular adaptations that reside within the muscle. Mechanical adaptations to EIMD suggest that remodelling of the intermediate filaments and/or an increase in intramuscular connective tissue provides protection against future bouts of muscle-damaging exercise. Friden et al. (1983) first proposed that an increase in cytoskeletal proteins or a structural reorganisation of the intermediate filament system might strengthen myofibrils to resist subsequent eccentric exercise. Cytoskeletal proteins, such as desmin and titin, are responsible for maintaining the structure and function of the sarcomere (Friden & Lieber, 1992). Studies have shown that considerable disruption and degradation occurs to desmin and titin proteins after EIMD (Friden & Lieber, 1998; 2001; Yu et al., 2003). Lieber et al. (1996) noted a loss in muscle fibre desmin content within 15 minutes of eccentric exercise in rabbits. Likewise, Trappe et al. (2002) reported a 30% reduction in titin content in human muscle after a single bout of muscle damage. However, there is evidence that an increase in cytoskeletal proteins can occur after initial EIMD (Friden et al., 1984; Baresh et al., 2002; Yu & Thornell, 2002; Yu et al., 2002; 2003; 2004; Lehti et al., 2007). Indeed, Yu and Thornell (2002) reported increased staining for both actin and desmin 2-8 days after

unaccustomed downstairs running. The authors suggested that this finding reflected an increase in protein synthesis, which might serve to withstand future muscle-damaging exercise. Similarly, Lehti et al. (2007) demonstrated that a single bout of eccentric exercise altered the staining patterns of titin, desmin and dystrophin. Increases in the mRNA levels of cytoskeletal proteins after acute eccentric exercise were suggested to provide protection against repeated bouts. Paulsen et al. (2009) observed an increase in desmin content in the cytoskeleton after initial and repeated bouts of EIMD. They speculated that rather than it being a one-time response to muscle damage, the upregulation of cytoskeletal proteins is a continuing adaptation to protect the muscle against repeated bouts of EIMD. Contradictory findings from Sam et al. (2000) posited that an increase in desmin content might lead to further muscle damage. When investigating the effects of muscle damage in mice with normal muscle and muscle lacking desmin, less damage was found in those mice lacking desmin. It was suggested that the absence of desmin made the muscle more compliant, enabling greater sarcomere shortening and lessening the strain along the myofibre. However, given that differences appear to occur to desmin content between species after EIMD (see Lieber et al., 1996; Yu et al., 2002), it is unknown if the absence of desmin prevents damage in human muscle.

Newham et al. (1987) suggested that the mechanism responsible for the RBE is associated with increased intramuscular connective tissue. It was proposed that any damage caused as a result of unaccustomed eccentric exercise might stimulate the synthesis of connective tissue, which acts to protect against further muscle damage. Clarkson and Tremblay (1988) also suggested that a strengthening of connective tissue explained the attenuation of muscle damage after repeated bouts of maximal eccentric exercise; however no supporting evidence was offered. Lapier et al. (1995)

provided indirect evidence that increased connective tissue enabled protection against muscle-damaging exercise. They examined intramuscular connective tissue in rat muscle immobilised for 3 weeks in either a shortened or lengthened position. Using tissue samples that were stained for collagen as an indicator of connective tissue, results indicated that muscles immobilised in the lengthened position had 63% more collagen, whilst muscle immobilised in the shortened position had 47% more connective tissue. Furthermore, eccentric exercise resulted in a 40% force loss in shortened muscle, whilst only an 8% decrement in force was observed in lengthened muscle. The protective effect was attributed to the increase in connective tissue being able to reduce myofibrillar stress during eccentric exercise. However, this finding could also reflect a cellular adaptation within the muscle fibre. That an effect was found when the muscle was immobilised in a lengthened position suggests that sarcomereogenesis occurred, which might have reduced sarcomere strain during the damaging bout (McHugh et al., 1999a; McHugh, 2003).

An increase in intramuscular connective tissue is also suggested to reflect an increase in passive muscle stiffness (McHugh et al., 1999a; McHugh, 2003). Indeed, it has been shown that eccentric training increases passive muscle stiffness, postulating that stiffness might also protect against EIMD (Reich et al., 2000; Brughelli & Cronin, 2007). However, contradictory research suggests that less muscle stiffness decreases the susceptibility to muscle damage (Nosaka & Clarkson, 1997; McHugh et al., 1999b). McHugh et al. (1999b) observed that those individuals categorised with stiffer hamstrings experienced greater symptoms of muscle damage in comparison to those with compliant hamstrings. More recently, Janecki et al. (2011) demonstrated a reduction in passive muscle stiffness after a second bout of

eccentric exercise, providing further evidence that an increase in passive muscle stiffness might not be responsible for the RBE.

The cellular theory proposes that the RBE is due to adaptations that occur at the site of the muscle fibre, the myofibril or the sarcomere (McHugh et al., 1999a). Armstrong (1984) proposed that the RBE was explained by the removal of weak muscle fibres that following recovery were replaced by stronger fibres; that were more resistant to repeated bouts of muscle damage. McHugh et al. (1999a) contended that the removal of 'susceptible' myofibrils or sarcomeres, as opposed to whole fibres, would be more consistent with the evidence of EIMD from biopsies. However, a limitation to the Armstrong et al. (1984) theory is that the initial bout of eccentric exercise does not have to result in EIMD to protect against a repeated bout. If weak sarcomeres were still intact after unaccustomed exercise, they should be disrupted by a repeated bout and result in EIMD (McHugh et al., 1999a). However, as aforementioned, studies have shown initial sub-maximal or low volume eccentric exercise that does not incur muscle damage protects against a subsequent maximal or high volume bout of EIMD (Brown et al., 1997; Chen et al., 2007a).

As mentioned earlier, strength loss post-EIMD is attributed to a combination of mechanical disruption to the myofibril and impairment in E-C coupling (Proske & Morgan, 2001). An adaptation in E-C coupling has been suggested to explain the attenuation in strength loss after a repeated bout of EIMD (McHugh et al., 1999a; McHugh, 2003). Clarkson and Tremblay (1988) speculated that strengthening of the sarcolemma or SR after initial EIMD could prevent the Ca^{2+} influx to the muscle cell and the ensuing E-C coupling failure following a repeated bout. However, the impairment of the E-C coupling is greatest immediately after EIMD (Ingalls et al.,

1998). Therefore, if the RBE was due to an adaptation in the E-C coupling, the reduction in strength immediately post-EIMD should be attenuated immediately after a repeated bout (McHugh, 2003). However, the loss in strength immediately post-eccentric exercise is shown to be similar between the initial and repeated bout of EIMD (Newham et al., 1987; Brown et al., 1997; McHugh, 2003).

Alternatively, Morgan (1990) hypothesised that after an initial bout of EIMD there is a longitudinal addition of sarcomeres, which allows the muscle to operate at longer lengths and reduces sarcomere strain during a repeated bout. The reduced strain then enables the myofilaments to maintain overlap, limits over-extending sarcomeres and avoids mechanical disruption (McHugh et al., 1999a). Lynn and Morgan (1994) and Lynn et al., (1998) provided evidence of an increase in sarcomereogenesis in rat muscle after eccentric exercise. Furthermore, the rightward shift in the length-tension relationship after unaccustomed eccentric exercise (see section 2.7.3) is also suggested to provide indirect evidence of a longitudinal addition of sarcomeres in humans. However, the rightward shift in optimum angle is only evident for 2 – 21 days after EIMD (Jones et al., 1997; Brockett et al., 2001; Bowers et al., 2004), which suggests that an increase in sarcomeregenesis is unable to explain the RBE that can last for 6 months after initial EIMD (Nosaka et al., 2001a).

Finally, alterations in the inflammatory response associated with EIMD have been attributed to the RBE. Studies have observed that inflammation in blood circulation is attenuated after a repeated bout of EIMD. Pizza et al. (1996; 2001) reported a significantly decreased neutrophil and monocyte response after a second bout of eccentric exercise. Smith et al. (2007) found a 50% decrease in IL-6 and a 10% reduction in monocyte chemoattractant protein-1 in circulation after repeated downhill running. They also reported a 95% increase in circulating anti-inflammatory

factor IL-10. Hirose et al., (2004) also observed a significant increase in IL-10 after repeated muscle-damaging exercise in the elbow flexors. An attenuated inflammatory response to repeated bouts of EIMD could reflect an adaptation to avoid the breakdown and removal of muscle tissue. Furthermore, an increase in anti-inflammatory response might serve to protect the muscle against pro-inflammatory cells following a repeated bout. However, the aforementioned studies investigated the effects of repeated bouts of EIMD on inflammatory factors in circulation and fail to examine inflammatory responses within the muscle. Stupka et al. (2001) observed that muscle counts of neutrophils and macrophages were significantly increased 24 h after a repeated bout of eccentric leg exercise. Similarly, Hubal et al. (2008) demonstrated that a number of inflammatory genes were up-regulated following a repeated bout of EIMD. However, CK response in the Stupka et al. (2001) study was significantly elevated 24 h after the repeated bout, which suggests that muscle damage was still evident after the second bout. The time span between bouts (5.5 weeks) might have also attenuated the level of protection conferred from the initial bout of EIMD (Byrnes et al., 1985). Although, indirect markers of EIMD were attenuated after Bout 2 in the Hubal et al. (2008) study, muscle biopsies were only taken 6 h after each bout of eccentric exercise.

In addition to inflammatory responses, several studies have examined the role in which heat shock proteins (HSP) can protect the muscle against EIMD (Thompson et al., 2001; 2002; Koh, 2002; Paulsen et al., 2007; 2009; Vissing et al., 2009). HSPs are up-regulated in response to stress and function to restore cellular homeostasis and protect against future insults (Morton et al., 2009). It is thought HSPs promote cellular recovery by binding with denatured proteins, before they irreversibly aggregate, and re-fold them to their original form (Paulson et al., 2007; Morton et al.,

2009). In response to muscle damage, HSP27, HSP70, α B-crystallin and ubiquitin have all been found to increase after initial and repeated bouts of eccentric exercise (Thompson et al., 2001; 2002; Stupka et al., 2001; Paulsen 2007; 2009). It is speculated that HSPs function to rescue disrupted sarcomeres and damaged proteins after unaccustomed exercise and function to protect and stabilise the muscle after a repeated bout (Paulsen et al., 2009). However, interpretation of studies undertaking repeated biopsies from the same muscle is difficult due to the risk of obtaining tissue that might have been affected by a previous biopsy. It is argued that the biopsy procedure itself may initiate a HSP response (Malm et al., 2001; McHugh, 2003).

It is unlikely that one underlying theory can explain the RBE due to the conflicting evidence against each theory (see McHugh et al., 1999a; McHugh, 2003). Instead, it is probable that the RBE occurs through an interaction of various neural and peripheral adaptations that are dependent on the mode and intensity of eccentric exercise adopted (McHugh et al., 1999a).

2.9 Physiological, metabolic and perceptual consequences of exercise-induced muscle damage during endurance performance

The effects of EIMD on markers of endurance performance remain equivocal and appear to be influenced by the method used to cause muscle damage, the mode of exercise adopted (i.e., cycling versus running), the exercise intensity, and the training status of participants (i.e., trained versus recreationally active).

2.9.1 The effects of exercise-induced muscle damage on oxygen uptake

Maximal oxygen uptake ($\dot{V}O_{2\max}$) appears to be unaltered after muscle-damaging exercise. Gleeson et al. (1998) reported no change in $\dot{V}O_{2\max}$ during incremental cycling following eccentric bench stepping. However, it remains to be seen if different modes of muscle-damaging exercise affect $\dot{V}O_{2\max}$ and/or whether $\dot{V}O_{2\max}$ is influenced by the mode of endurance exercise after EIMD.

Exercise-induced muscle damage does appear to affect oxidative metabolism via two contrasting pathways; either sub-maximal $\dot{V}O_2$ for a given exercise intensity is unaltered (Gleeson et al., 1995; Walsh et al., 2001; Moysi et al., 2005; Davies et al., 2009; Twist & Eston, 2009), or it increases after muscle-damaging exercise (Kyrolainen et al., 2000; Calbet et al., 2001; Braun & Dutto, 2003; Chen et al., 2007b; 2009). Gleeson et al. (1995) found no significant difference in sub-maximal $\dot{V}O_2$ during fixed-intensity cycling at 80% $\dot{V}O_{2\max}$ after eccentric bench stepping, despite significant increases in muscle soreness and serum CK activity. More recently, Twist and Eston (2009) reported no change in $\dot{V}O_2$ during fixed-intensity cycling bouts at 60 and 80% $\dot{V}O_{2\max}$ after muscle-damaging plyometrics. These findings were supported by Davies et al. (2009), who reported the $\dot{V}O_2$ response during sub-maximal cycling at 80% of the gas exchange threshold (GET) to be unchanged after EIMD caused by resistance exercise (100 squats at 70% body mass). Conversely, Kyrolainen et al. (2000) demonstrated that after marathon running (which generated symptoms of EIMD) there was a significant increase in $\dot{V}O_2$ response during subsequent sub-maximal running performance. Likewise, Braun and Dutto (2003)

also reported an elevation in $\dot{V}O_2$ across three sub-maximal running intensities (65, 75 and 85% $\dot{V}O_{2\max}$) after a bout of downhill running. The contrasting findings have led to speculations that after muscle damage the $\dot{V}O_2$ response is dependent on the mode of endurance exercise selected. As highlighted above, studies showing no change in sub-maximal $\dot{V}O_2$ have typically utilised cycling. Therefore, it is postulated that alterations in limb kinematics (stride pattern) after EIMD will impose a greater impact on running compared to cycling, especially given that limb kinematics would be expected to show minimal, if any deviation during cycling. Therefore, it is unlikely that oxygen cost will increase after muscle damaging exercise (Jones & Burnley, 2005). One study has observed an increase (3%) in sub-maximal $\dot{V}O_2$ during cycling after muscle-damaging exercise (Burt & Twist, 2011); however this finding was probably due to the participants being unfamiliar with cycling exercise.

Several studies have shown that EIMD provokes significant changes to running kinematics (Hamill et al., 1991; Kyrolainen et al., 2000; Braun & Dutto, 2003; Paschalis et al., 2007a; Chen et al., 2007b; 2009). Chen et al. (2007b) reported significant reductions in stride length, ranges of motion of the ankle and knee joints, and a significant increase in stride frequency during sub-maximal running at 85% $\dot{V}O_{2\max}$ following eccentric exercise. Moreover, researchers have associated these alterations in gait kinematics to increases in $\dot{V}O_2$ (Braun & Dutto, 2003; Chen et al., 2007b; 2009). For example, Braun and Dutto (2003) reported a negative correlation ($r = -0.535$, $P < 0.05$) between the change in $\dot{V}O_2$ during running after muscle damage with the change in stride length. Furthermore, Cavanagh and Williams (1982) have shown that when a runner deviates (reduces or increases) from their optimal stride length, subsequent increases in oxygen cost occur. Concomitant

increases in muscle soreness as a result of eccentric exercise make it possible to speculate that participants may change their running pattern in order to limit their discomfort.

Kyrolainen et al. (2000) attributed increased $\dot{V}O_2$ responses during sub-maximal running to alterations in neuromuscular function. They suggested that impaired neuromuscular control after muscle-damaging exercise required additional motor unit activation in order to produce the same resultant force during fixed-intensity endurance exercise. Semmler et al. (2007) recently supported this hypothesis, observing that reductions in MVC force after EIMD coincided with increases in EMG activity during sub-maximal exercise. Previously, Bigland-Ritchie and Woods (1974) had demonstrated a linear relationship between EMG activity and oxygen consumption, providing indirect evidence that alterations in oxygen metabolism during sub-maximal endurance exercise after EIMD might be due to an increase in motor unit activity. However, this explanation has remained uncorroborated and requires further investigation.

The difference in $\dot{V}O_2$ response between running and cycling exercise after muscle damage could also be due to alterations in the SSC. In contrast to cycling, running utilises the SSC to provide elastic energy during repeated eccentric and concentric actions (Komi, 2000; Jones & Burnley, 2005), and whilst long-term SSC exercise increases muscle stiffness and subsequent running economy (see Section 2.1), unaccustomed SSC activity has been shown to reduce muscle stiffness (Nicol et al., 1996; Horita et al., 1996; 1999). Avela and Komi (1998) reported that decreased muscle performance after marathon running was associated with a decline in muscle stiffness and subsequent inability to utilise elastic energy. Adopting this explanation,

Chen et al. (2007b; 2009) attributed decrements in running economy to an impaired ability to absorb and utilise elastic energy caused by muscle-damaging exercise. However, whilst this appears to be a credible explanation to explain increases in $\dot{V}O_2$, no changes in musculotendinous stiffness during running after muscle damage were reported.

Not all of the studies investigating the effects of EIMD on running performance have observed an increase in $\dot{V}O_2$ (Scott et al., 2003; Paschalis et al., 2005; 2008b; Marcora & Bosio, 2007). Despite evidence of muscle damage after eccentric exercise, Paschalis et al. (2005) failed to report concomitant changes in $\dot{V}O_2$ during selected sub-maximal running intensities. Similarly, a further study by Paschalis et al. (2008b) reiterated that eccentric exercise did not affect $\dot{V}O_2$ response during sub-maximal running performance. Interestingly though, both of these studies recruited only recreational athletes. Research that has recruited well-trained runners and observed alterations in oxygen metabolism postulate that $\dot{V}O_2$ response to EIMD is due to trained runners' having a more refined gait pattern (Braun & Dutto, 2003). Whilst this is an interesting argument, no study has yet to examine responses to muscle damage during running between trained and untrained athletes to identify if differences in gait pattern occur. Moreover, there are studies using trained runners that have reported no change in running $\dot{V}O_2$ response after muscle-damaging exercise. For instance, Scott et al. (2003) observed no alteration in $\dot{V}O_2$ responses to sub-maximal running after a bout of lower body resistance exercises designed to elicit symptoms of muscle damage. Similarly, Marcora and Bosio (2007) also reported no significant increase in $\dot{V}O_2$ during running in a mixed group of runners and triathletes after muscle-damaging plyometrics. However, it is noteworthy that in

both of these studies, the sub-maximal running intensity used to examine $\dot{V}O_2$ response to EIMD was equal to or below 70% $\dot{V}O_{2\max}$, leading the researchers to suggest that the effects of EIMD on running $\dot{V}O_2$ response is also dependent on the exercise intensity selected. To examine this hypothesis, Chen et al. (2009) examined $\dot{V}O_2$ responses to running at 70, 80, and 90% $\dot{V}O_{2\max}$ after a period of downhill running. Significant changes in $\dot{V}O_2$ from baseline were evident at 48 h and 120 h after eccentric exercise at 80 and 90% $\dot{V}O_{2\max}$. However, $\dot{V}O_2$ remained unchanged during running at 70% $\dot{V}O_{2\max}$, confirming that $\dot{V}O_2$ during high intensity running is more affected than at low intensity after muscle damage.

While exercise modality, exercise intensity and training status are important determinants of the responses to sub-maximal exercise after EIMD (Davies et al., 2009), the mode of muscle-damaging exercise also appears to affect $\dot{V}O_2$ responses to endurance exercise, particularly during running. For example, studies that report increases in $\dot{V}O_2$ during sub-maximal running tend to use downhill running to induce muscle damage (Braun & Dutto, 2003; Chen et al., 2007b; 2009). Moreover, irrespective of training status, exercise intensity and significant changes in the markers of EIMD, studies that observe a non-significant change in $\dot{V}O_2$ during running use contrasting modes of muscle-damaging exercise, such as plyometrics (Marcora & Bosio, 2007), isokinetic eccentric exercise (Paschalis et al., 2005; 2008b) or lower limb resistance exercise (Scott et al., 2003). However, to date there is no study to confirm whether different modes of muscle damage induce contrasting responses in $\dot{V}O_2$ during sub-maximal running.

2.9.2 Muscle oxygenation

Recent research has observed that $\dot{V}O_2$ kinetics (the rate at which oxygen uptake increases at the onset of exercise) remain unchanged after muscle-damaging exercise (Davies et al., 2008; Schneider et al., 2007). This appears surprising given that the inflammatory response during the degeneration and regeneration phases of the muscle fibre after EIMD could prevent muscle microcirculation, leading to an imbalance between muscle oxygen delivery and oxygen utilisation (Davies et al., 2008; Ahmadi et al., 2008; Schneider et al., 2007; Walsh et al., 2001). Oxygen kinetics is postulated to reflect the ability of oxygen transport and utilisation, therefore impairment in oxygen delivery and utilisation could potentially retard the $\dot{V}O_2$ kinetic response (Poole et al., 2008; Schneider et al., 2007). In particular, Davies et al. (2008) hypothesised that EIMD could lead to individuals crossing the oxygen “tipping point” (Poole et al., 2008) in which muscle oxygen availability becomes limited and inevitably leads to a decrement in $\dot{V}O_2$ kinetic response. However, Davies et al. (2008) found that the $\dot{V}O_2$ kinetic response was not slowed as a result of muscle damage. Moreover, Schneider et al. (2007) concluded that muscle-damaging exercise failed to impair oxygen delivery or reduce oxygen utilisation during high-intensity exercise. However, Schneider et al. (2007) were unable to support this statement due to their failure to measure muscle oxygenation during endurance performance (Davies et al., 2008). Despite this, Walsh et al. (2001) have reported that muscle oxygenation measured via near infrared spectroscopy (NIRS) failed to change after eccentric cycling. However, these findings must be viewed with caution since the authors themselves questioned whether the muscle-damaging exercise they adopted was sufficient enough to induce symptoms of EIMD. Conversely, Ahmadi et al. (2008) demonstrated that

downhill walking led to an immediate increase in resting muscle oxygen utilisation, along with oxygen de-saturation and re-saturation kinetics that were significantly faster than baseline values. These changes were attributed to impairments in microcirculatory flow, increases in slow-twitch fibre recruitment and an increased requirement for aerobic energy-demanding repair processes. More recently, Davies et al. (2008) using NIRS indicated that de-oxyhemoglobin response was significantly slower after muscle-damaging exercise. They postulated that the balance between oxygen delivery and utilisation might have been increased in order to preserve an unchanged $\dot{V}O_2$ kinetic response. Moreover, it was suggested that the participants might have been operating to the right of the oxygen “tipping point” during cycling endurance exercise in order to prevent a slowing of $\dot{V}O_2$ kinetics after EIMD (Davies et al., 2008; Poole et al., 2008). Whilst it appears $\dot{V}O_2$ kinetic response is unchanged after muscle-damaging exercise during cycling endurance exercise, no study to date has investigated whether this response is consistent across other modes of endurance exercise, such as running. This is particularly pertinent given the differences typically seen in $\dot{V}O_2$ kinetic response between running and cycling exercise (Jones & McConnell, 1999; Carter et al., 2000; Hill et al., 2003).

2.9.3 Ventilatory responses to exercise

There is increasing evidence demonstrating that the \dot{V}_E response during sub-maximal exercise increases as a result of EIMD (Gleeson et al., 1995; Braun & Dutto, 2003; Hotta et al., 2006; Chen et al., 2007b; 2008; 2009; Davies et al., 2008; 2009; Twist & Eston, 2009). Moreover, whilst exercise modality appears to be an important factor in determining $\dot{V}O_2$ response to endurance exercise after EIMD,

ventilation does not appear to be dependent on the mode of endurance exercise selected, with significant increases in the \dot{V}_E response observed during cycling (Gleeson et al., 1995; Davies et al., 2008; 2009; Twist & Eston, 2009) and running (Chen et al., 2007b; 2008) after eccentric exercise. Exercise intensity is also unlikely to influence ventilation after muscle-damaging exercise. Davies et al. (2009) demonstrated that after a bout of squatting exercise significant increases in the \dot{V}_E response occurred during both moderate (80% of the GET) and severe (70% of the difference between the GET and $\dot{V}O_{2\max}$) intensity cycling. Likewise, Twist and Eston (2009) reported increased \dot{V}_E responses at 60 and 80% of the power output corresponding to $\dot{V}O_{2\max}$.

Paschalis et al. (2005; 2008b) observed no change in \dot{V}_E during sub-maximal running after a bout of isokinetic eccentric exercise, suggesting that the mode of muscle damaging exercise adopted might be an important factor in determining \dot{V}_E response to endurance exercise. In particular, these findings could be due to the specific muscle recruitment patterns of isokinetic exercise, which would primarily result in muscle damage to the quadriceps and only limited effects on the other lower limb musculature typically recruited during cycling and running exercise, such as the biceps femoris, gastrocnemius, and gluteus maximus (Bijker et al., 2002). In studies which have shown significant increases in ventilation the modes of muscle-damaging exercise have involved multi-joint movements, such as; downhill running (Chen et al., 2007b), squatting (Davies et al., 2008) and plyometrics (Twist & Eston, 2009). Such exercises would be expected to recruit and damage a greater range of lower limb musculature that would explain the greater ventilatory response during sub-maximal cycling and running exercise.

Several mechanisms have been provided to explain the observed elevations in sub-maximal \dot{V}_E response following EIMD, including reduced oxygen economy (Chen et al., 2007b), increased acidosis (Gleeson et al., 1995; Braun & Dutto, 2003), and increased muscle afferent discharge (Davies et al., 2008; Twist & Eston, 2009). Chen et al. (2007b) suggested that changes in \dot{V}_E during sub-maximal running were indicative of a reduction in running economy as a result of muscle damage. It is reasonable to assume that an increased respiratory response occurred to facilitate an elevated oxygen cost. However, several studies have reported significant increases in \dot{V}_E response without contemporaneous rises in $\dot{V}O_2$ (Gleeson et al., 2005; Davies et al., 2009; Twist & Eston, 2009), suggesting that other mechanisms might be involved. Indeed, it has been speculated that the ventilatory response is augmented by the respiratory system to offset elevations in blood lactate concentration ([La]) after muscle-damaging exercise. Blood lactate concentration has been shown to be increased during sub-maximal exercise after a prior bout of damage-inducing exercise (Gleeson et al., 1995; 1998; Braun & Dutto, 2003; Chen et al., 2007b; Schneider et al., 2007). It is posited that \dot{V}_E responses are increased in order to excrete additional carbon dioxide as a result of elevated [La] during endurance exercise after muscle damage (Gleeson et al., 1995). However, Davies et al. (2009) and Twist and Eston (2009) have both observed significant elevations in \dot{V}_E during moderate and severe intensity endurance exercise after EIMD without concomitant increases in [La]. Twist and Eston (2009) also found that the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) was increased; indicating that \dot{V}_E during exercise was not elevated to counteract rises in $\dot{V}CO_2$ after EIMD. Furthermore, it is possible to dissociate [La] from \dot{V}_E during exercise in McArdle's disease patients

(Hagberg et al., 1982). Hagberg et al. (1982) still found increases in \dot{V}_E during exercise in McArdle's disease patients, despite their inability to produce lactate due to a lack of muscle phosphorylase.

Ventilatory responses during exercise are controlled through two distinctly separate mechanisms (Amann, 2012). The first is a feed-forward mechanism, termed 'central command', and is posited to increase \dot{V}_E by activating the insular or medial prefrontal cortex (Amann et al., 2011). It is possible that increases in central command, to ensure the same force during endurance exercise is maintained as a result of decrements in muscle function after EIMD, activate regions of the brain that control \dot{V}_E response. Alternatively, the second is a feedback mechanism that reflexly augments \dot{V}_E as a consequence of muscular contraction (Amann et al., 2011; Amann, 2012). Group III and IV afferent fibres, located in and around the blood vessels of the exercising musculature, are also suggested to be involved in controlling \dot{V}_E response (Hotta et al., 2006). Davies et al. (2008) and Twist and Eston (2009) proposed that elevations in \dot{V}_E during endurance exercise are due to alterations in muscle afferent activity after EIMD. It is suggested that EIMD provokes a discharge from muscle afferents that is projected via the spinal cord to various sites in the CNS; resulting in an increase in \dot{V}_E (Davies et al., 2008; 2009; Twist & Eston, 2009; Amann, 2012). Amann et al. (2010) provided evidence that muscle afferents facilitate \dot{V}_E response during exercise. They observed that pharmacologically blocking the central projection of Group III and IV afferents significantly reduced \dot{V}_E during sub-maximal cycling.

2.9.4 Metabolic responses to exercise

Increases in [La] during endurance exercise would suggest that muscle metabolism is altered as a result of muscle damage. Indeed several studies have reported significant elevations in both sub-maximal and peak [La] during endurance exercise after EIMD (Gleeson et al., 1995; 1998; Braun & Dutto, 2003; Schneider et al., 2007; Chen et al., 2007b; 2008; 2009). It is postulated that impaired muscle metabolism is attributed to a reduction in oxygen extraction in the blood by the working musculature as a result of muscle damage, leading to an increased reliance on anaerobic metabolism to produce sufficient ATP. However, Gleeson et al. (1998) observed that $\dot{V}O_{2\text{ peak}}$ was unaffected after muscle-damaging exercise despite significant elevations in peak [La] being evident. Furthermore, Schneider et al. (2007) also reported that elevated sub-maximal [La] was not accompanied by significant alterations in phase II oxygen uptake kinetic response after EIMD. It was concluded that the increased [La] was not due to alterations in oxidative function. The authors speculated that higher [La] during sub-maximal cycling was due to an increased rate of lactate efflux from the exercising muscles (due to an enhanced muscle membrane permeability) after muscle-damaging exercise. However, Davies et al. (2009), who reported an unchanged [La] response after EIMD, suggested that a higher rate of lactate efflux could result in an enhanced clearance that is facilitated by the increased muscle blood flow due to muscle damage (Laaksonen et al., 2006). Alternatively, Gleeson et al. (1995; 1998) attribute elevated [La] to a greater metabolic strain on undamaged muscle fibres after EIMD. It is possible that preferential damage to type II fibres after EIMD (Friden et al., 1983) leads to an increased recruitment of non-damaged type II fibres in order to maintain pre-damage force capacities during fixed-intensity endurance exercise (Gleeson et al., 1998; Braun & Dutto, 2003; Chen et al., 2007b).

Since type II fibres are more glycolytic, an increase in their activation following muscle damage might result in an increased [La] response during endurance exercise (Gleeson et al., 1998; Scott et al., 2003). However, the observed increase in [La] following eccentric exercise may only be evident during higher intensities (Twist & Eston, 2009). Indeed, Scott et al. (2003) reported that [La] failed to alter significantly after muscle-damaging exercise during running at an intensity deemed to be below the lactate threshold (LT). The authors suggested that because type I muscle fibres are recruited at intensities below the LT and are less susceptible to muscle damage (Friden et al., 1983), they may have been able to maintain a normal recruitment pattern and metabolic function (Scott et al., 2003). Moreover, Chen et al. (2009) recently investigated metabolic responses to running at exercise intensities corresponding to below, equal to, and above the LT following a period of downhill running. Significant changes in [La] were evident at and above the LT, whilst metabolic responses remained unchanged during running below the LT. Coupled with those of Scott et al. (2003), these findings suggest that [La] after muscle damage is dependent upon the intensity of endurance exercise adopted.

2.9.5 Glycogen metabolism

It is well documented that muscle glycogen is a vital fuel source for the maintenance of successful endurance performance (Burke, 2001). However, a number of investigations have observed that glycogen metabolism is impaired as a result of muscle damage, thereby posing a threat to endurance capacity (Sherman et al., 1983; O'Reilly et al., 1987; Costill et al., 1990; Asp et al., 1995a; 1997; 1998; 1999).

Attempts to clarify the mechanism behind why glycogen metabolism is impaired after muscle-damaging exercise have tended to focus on the action of GLUT-4 protein

concentration and inflammatory responses to EIMD (Tee et al., 2007). As GLUT-4 is the most abundant glucose transporter in skeletal muscle, any reduction in GLUT-4 content as a result of muscle damage could impair glycogen resynthesis within the muscle cell membrane (Asp et al., 1995a; 1995b; 1998; Tee et al., 2007). However, alterations in GLUT-4 concentration after muscle-damaging exercise appear equivocal and dependent upon the training status of the participants recruited and the mode of muscle damage selected. Asp et al. (1995a) initially observed a 17% decrease in GLUT-4 content amongst a group of untrained males after eccentric cycling exercise, and in a later study (Asp et al., 1997) demonstrated that GLUT-4 response in well-trained males after marathon running was unchanged. This was despite an increased CK activity and reduced muscle glycogen concentration being present 48 h after damage-inducing exercise. Alternatively, it has been proposed that impaired glycogen metabolism as a result of EIMD is due to the infiltration of inflammatory cells competing with muscle fibres for the available plasma glucose (Costill et al., 1990; Asp et al., 1995a; Tee et al., 2007). However, Asp et al. (1995b) observed significant reductions in glycogen content after EIMD without concomitant increases in inflammatory response, therefore providing evidence that accumulation of inflammatory cells is unlikely to explain the suppressed glycogen concentration after EIMD.

Although impaired muscle glycogen resynthesis after eccentric exercise is well documented and poses a threat to endurance capability (Sherman et al., 1983; O'Reilly et al., 1987; Costill et al., 1990; Asp et al., 1995a; 1995b; 1997; 1998; 1999), the process involved has not been established. Therefore, future research is warranted to establish how glycogen metabolism is altered as a result of muscle-damaging exercise (Tee et al., 2007).

2.9.6 Perceptual responses to exercise

Whilst physiological and metabolic responses to eccentric exercise appear to be influenced by a number of factors, several studies, irrespective of muscle damage modality, participant training status and endurance exercise mode, have demonstrated that the rating of perceived effort (RPE) is increased after EIMD (Gleeson et al., 1995; Asp et al., 1998; Scott et al., 2003; Marcora & Bosio, 2007; Chen et al., 2007b; 2008; 2009; Davies et al., 2008; 2009; Twist & Eston, 2009; Burt & Twist, 2011). Hampson et al. (2001) suggested that RPE involves a combination of numerous sensory cues derived from both peripheral and central factors. Similarly, Jameson and Ring (2000) have suggested that RPE during endurance exercise is based upon an integration of increased leg pain and feelings of breathlessness. Therefore, augmented muscle soreness as a result of EIMD might be a determinant of RPE during endurance exercise (Davies et al., 2009). Moreover, increased physiological responses after muscle damage are also likely to provide a central cue to inform RPE during endurance exercise. Indeed, Robertson (1982) speculates that there is a critical metabolic rate at which ventilation influences overall RPE. It is postulated that as the ventilatory response increases, after EIMD, activated mechanoreceptors within the chest wall, lungs and airways influence the participant in perceiving breathing rate to be harder, leading to the elevation of RPE response during endurance exercise (Hampson et al., 2001). Work from Amann et al. (2010) provided indirect evidence that elevated ventilatory response during endurance exercise influences RPE. They observed that blocking afferent feedback responses from locomotor musculature during exercise resulted in the reduced ventilation response coincided with a reduction in RPE.

Marcora (2009) challenged the concept that sense of effort during exercise is based on peripheral mechanisms. In an earlier study, Marcora et al. (2008) observed that 40 minutes after EIMD, increases in RPE during cycling occurred despite no evidence of muscle soreness. They suggested that RPE is the conscious awareness of the central motor command sent to the exercising muscle; termed the 'sensation of innervation'. Central motor command is the activity of premotor and motor areas of the brain related to voluntary muscular contraction (Marcora, 2009). It is posited that a corollary discharge or efference copy of the central motor command is forwarded to the sensory areas of the brain to generate RPE (Marcora, 2009).

Stauber (1989) suggested that after EIMD the central nervous system evokes an alternative motor control strategy to distribute force production over a larger pool of muscle fibres. It was this increase in muscle fibre recruitment, required to produce the same baseline running speed after EIMD, that Scott et al. (2003) posited was responsible for an increase in RPE. In support, Elmer et al. (2010) suggested that an increase in central motor command, to enable the same pre-EIMD power during sub-maximal cycling, resulted in the greater RPE. de Morree et al. (2012) examined the relationship between RPE and central motor command during a weight raising task. Participants were required to raise a light and heavy weight with a fatigued and non-fatigued arm, during which RPE and movement-related cortical potential (MRCP) were recorded. Movement-related cortical potential measures the activity of premotor and motor areas of the brain and therefore provides a direct measure of central motor command (de Morree et al., 2012). They observed that RPE and MRCP during the weight raising task increased with weight and muscular fatigue. Furthermore, a significant correlation between RPE and MRCP across both experimental conditions was found; lending support to the corollary discharge theory

of effort perception. However, in a force-matching task in which the force produced in a damaged and non-damaged arm was perceived to be the same, the EMG activity was greater in the damaged arm (Carson et al., 2002). This suggests that effort perception is not based on corollary discharge from the motor cortex. Carson et al. (2002) indicated that the relationship between RPE and central motor command is altered after EIMD, and proposed that RPE is generated from neural centres upstream from the motor cortex.

It is unlikely that an afferent or efferent pathway is solely responsible for RPE during exercise. Instead, it is probable that RPE is dependent on an interaction of both peripheral and central mechanisms (Smirmaul, 2012). Indeed, the 'reafferent corollary discharge' theory suggests that RPE is generated via a central signal that traverses efferent and then afferent pathways to feed the sensory areas of the brain (Luu et al., 2011; de Morree et al., 2012). For example, increased central motor command elevates muscle activation to ensure the same pre-EIMD force is achieved during endurance exercise. The subsequent increase in muscle activation augments muscle spindle firing, which is then relayed to the sensory areas of the brain, where it is used to generate RPE. However, this theory is not confirmed and future research to establish the contribution of the afferent and efferent pathways on RPE is required (de Morree et al., 2012).

2.10 The implications of muscle-damaging exercise on time-trial performance

A bout of fixed intensity exercise continuing until volitional exhaustion is the most commonly used assessment of endurance capacity (Jeukendrup et al., 1996; Schabert et al., 1998; Currell & Jeukendrup, 2008). However, whilst such open-loop protocols provide the opportunity to measure physiological responses during steady

state exercise, they appear to have low ecological validity (Schabert et al., 1998; Currell & Jeukendrup, 2008; Sewell & McGregor, 2008) and poor reliability (Krebs & Powers, 1989; McLellan et al., 1995; Jeukendrup et al., 1996). Alternatively, the use of simulated time-trials, whereby participants complete a fixed amount of work or cover a set distance in the shortest time possible provide a more reliable and accurate measurement of endurance performance (Foster et al., 1993; Jeukendrup et al., 1996; Lindsay et al., 1996; Palmer et al., 1996; Bishop et al., 1997; Schabert et al., 1998).

The utility of these closed-loop protocols also enables the effects of muscle damage to be examined in a setting that is indicative of how endurance athletes typically compete. Marcora and Bosio (2007) have investigated the effects of plyometric muscle-damaging exercise on 30-minute running time-trial performance. It was observed that whilst physiological responses were unaltered, average running speed and distance covered were significantly lower after EIMD. More recently, Twist and Eston (2009) and Burt and Twist (2011) examined the effects of muscle damage on cycling time-trial performance, and demonstrated that power output, distance covered, and physiological and metabolic responses were significantly reduced 48 h after EIMD induced by plyometric exercise.

Notwithstanding the observed decrease in time-trial performance, perceptual responses appear to be unchanged after EIMD (Marcora & Bosio, 2007; Twist & Eston, 2009; Burt & Twist, 2011). However, this suggests that in a muscle damaged state RPE is greater for a lower exercise intensity and physiological and metabolic cost. It is postulated that changes in performance after muscle-damaging exercise are attributed to an altered sense of effort and not damage to skeletal muscle per se (Miles et al., 1997; Carson et al., 2002; Proske et al., 2003; Weerakkody et al.,

2003). However, if an altered sense of effort is responsible for impaired time-trial performance, the observed reduction in physiological response after EIMD is unlikely to provide a central cue to explain why perceptual response is altered.

Several studies have reported that after fatiguing exercise, individuals are capable of quantitatively scaling their effort in an attempt to internally regulate exercise intensity in the face of disturbed homeostasis (Eston et al., 2007; Crewe et al., 2008). Twist and Eston (2009) postulate that the central nervous system might reduce the neural drive to the peripheral muscles during a time-trial after muscle damage to protect against further injury. It is possible that despite the conscious effort of the participants to cover as much distance as possible, muscle fibres may have been “spared” during the time-trial performance after muscle damage as part of a protective mechanism by the subconscious brain to prevent further injury (Burt & Twist, 2011). However, whilst this is an interesting concept it is yet to be confirmed.

Alternatively, Marcora and Bosio (2007) hypothesise that the inflammatory response after EIMD is a possible mechanism behind the reduction in exercise tolerance. Cytokines, such as IL-1, are primary mediators behind the muscle’s inflammatory response to EIMD (Tidball, 1995). Once evident in the muscle, it is speculated that IL-1 can enter circulation whereby it has the potential to affect distant organs such as the brain (Carmichael et al., 2005). Interestingly, Rinehart et al. (1997) and Omdal and Gunnarsson (2005) have found that increased levels of IL-1 in the brain causes symptoms of fatigue in human participants. Indeed, Carmichael et al. (2005) attributed decrements in endurance performance after EIMD in mice to elevated concentrations of IL-1 in the cortex and cerebellum regions of the brain. While this mechanism has been suggested to explain impaired time-trial performance in cyclists (Burt & Twist, 2011), it has, to date, only been confirmed in animal models.

Therefore future research investigating the relationship between IL-1 activity in the brain and exercise tolerance after EIMD among human participants is warranted.

Although the effects of EIMD have been shown to impair endurance performance that is more indicative of how athletes compete in a real world scenario, the aforementioned studies are limited to relatively short-term (i.e. 5 – 30-minute) time-trial performance, the recruitment of recreational active participants, and the adoption of laboratory-based protocols. Future research is warranted investigating the impact of muscle-damaging exercise on endurance performance during event-specific distances in a field-based environment among well-trained athletes.

2.11 The effects of exercise-induced muscle damage on resting metabolic rate

A body of research has emerged demonstrating that the effects of EIMD can increase resting metabolic rate (RMR) for up to 72 h post-exercise (Dolezal et al., 2000; Schuenke et al., 2002; Jamurtas et al., 2004; Hackney et al., 2008; Paschalis et al., 2010). Investigating the consequence of a single bout of whole body resistance exercise (6 sets of 10 upper and lower body exercise at 70% 1 repetition maximum; RM), Melby et al. (1993) reported that RMR was still significantly elevated at 15 h post-exercise. Likewise, Gillette et al. (1994), adopting a similar resistance exercise protocol, demonstrated that RMR was increased above baseline values for up to 14.5 h. It was not the aim of these studies to investigate the effects of EIMD on post-exercise RMR, although it was speculated that the eccentric muscle actions associated with the resistance exercise might have induced muscle damage and consequently increased RMR. Furthermore, in both studies resting $\dot{V}O_2$ had not returned to baseline and were pre-determined times set by the investigators, which suggests that the time course of the impact EIMD on RMR might be longer.

Dolezal et al. (2000) investigated whether muscle damage, resulting from an acute bout of lower limb resistance exercise (8 sets of leg presses at 6RM), could influence RMR. RMR was significantly elevated for up to 48 h post-exercise and occurred alongside increases in CK and DOMS to support speculation that EIMD alters resting metabolism. Schuenke et al. (2002) reported elevations in resting $\dot{V}O_2$ for up to 38 h after a single bout of whole body resistance exercise; however no markers of EIMD were taken to suggest that damage to the muscle contributed to the increase in RMR. Jamurtas et al. (2004) reported that CK, DOMS and RMR were significantly increased for up to 24 h after 60 min of resistance exercise. Similarly, Hackney et al., (2008) observed significant elevations in CK, DOMS and RMR for up to 72 h after whole body resistance exercise. Paschalis et al. (2010) also demonstrated that EIMD increased RMR for up to 72 h. Furthermore, in an extension of this study, Paschalis et al. (2011) reported that acute and chronic eccentric exercise caused increases in RMR. RMR was shown to be increased by 12.7% in the 48 h after the acute bout of eccentric exercise and was 5% higher after 8 weeks of eccentric training.

Williamson and Kirwan (1997) evaluated the effects of an acute bout of concentric resistance exercise on resting metabolism and observed a 3% increase at 48 h after exercise. However, this is considerably lower than the 5.5 – 25% increase in RMR observed 24 – 72 h after eccentric-biased exercise (Dolezal et al., 2000; Jamurtas et al., 2004; Hackney et al., 2008; Paschalis et al., 2010). These findings confirm that the increased metabolic rate in the days after a single bout of resistance exercise is dependent upon the involvement of eccentric muscle contractions. Kolkhorst et al. (1994) and Thomas et al. (1994) failed to report any increase in RMR 24 h after level and downhill running. However, both studies failed to assess the extent of muscle damage, which undermines the significance of their findings (Dolezal et al., 2000).

There are several mechanisms posited to explain why RMR is elevated after exercise, such as the resynthesis of glycogen from lactate, increased body temperature, replenishment of oxygen stores in blood and muscle, resynthesis of ATP and creatine phosphate (CP), increased circulation and ventilation and residual hormone effects (Gaesser & Brooks, 1984; Bahr, 1992; Borsheim & Bahr, 2003). However, these mechanisms are associated with the rapid and slow components of the excess post-exercise oxygen consumption (EPOC), which might only persist for a few hours, and are unlikely to contribute to the prolonged increase in RMR that occur for up to 72 h post-EIMD (Dolezal et al., 2000; Hackney et al., 2008).

Alternatively, the prolonged increase in RMR observed after an acute bout of eccentric-biased exercise is attributed to the appearance of muscle damage and the stimulus for repair (Burleson et al., 1998; Dolezal et al., 2000; Paschalis et al., 2010). EIMD is accompanied by an inflammatory response that causes the influx of neutrophils and macrophages to the site of injury (Smith, 1991; MacIntyre et al., 1995; Sorichter et al., 1999), the aim of which is to promote the degradation and synthesis of damaged muscle fibres (MacIntyre et al., 1995). Chesley et al. (1992) reported that muscle protein synthesis was significantly increased for up to 24 h after a single bout of resistance exercise. In rat tibialis anterior muscle, Wong and Booth (1990) observed that muscle protein synthesis was increased by 45% as a result of muscle-damaging eccentric exercise. Welle and Nair (1990) found that the energy cost of protein synthesis accounts for as much as 20% of total resting metabolism. Furthermore, protein synthesis has also been shown to correlate positively with oxygen uptake and RMR in adults with severe burn trauma (Cunningham et al., 1989).

A prolonged increase in RMR after muscle damage is also attributed to an elevation in sympathetic nervous system activity (Scheunke et al., 2002; Jamurtas et al., 2004). The release of norepinephrine promotes $\dot{V}O_2$ by stimulating the dilation of blood vessels that supply skeletal muscle (Sherwood, 2001; Schuenke et al., 2002). Moreau et al. (1995) found increased concentrations of urinary norepinephrine and epinephrine 24 – 48 h after EIMD. The authors posited that the stress associated with movement of sore muscles led to the release of catecholamines from the adrenal medulla.

Concomitant with elevations in RMR post-EIMD, studies have shown that fat utilisation is increased in the days after muscle-damaging exercise (Schuenke et al., 2002; Jamurtas et al., 2004; Paschalis et al., 2010; 2011). Using respiratory quotient (RQ) to provide an indirect measure of substrate use, a decrease in RQ suggests that fat utilisation is increased. EIMD has been found to increase insulin resistance (Tee et al., 2007), reduce glucose disposal rates (Kirwan et al., 1992) and decreases GLUT-4 protein concentration (Asp et al., 1995). Therefore, it is plausible that changes to the glucose transport system might explain the reliance on fat during recovery from EIMD (Paschalis et al., 2010). Damage to the muscle membrane could also breakdown fatty acid phospholipids, increasing beta oxidation due to the greater availability of free fatty acid inside the muscle cell (Paschalis et al., 2010). Alternatively, the increased reliance on fat during recovery from EIMD could be due to the inflammatory cells, associated with muscle damage, competing with muscle fibres for available plasma glucose (Costill et al., 1990; Asp et al., 1995; Jentjens & Jeukendrup, 2003; Tee et al., 2007).

Finally, given that RMR accounts for the largest percentage (65 – 75%) of total daily energy expenditure (Dolezal & Potteiger, 1998), any increase in RMR as a result of EIMD could have implications for athletes attempting to maintain a positive energy balance to facilitate hypertrophy and for those individuals attempting a negative energy balance to promote weight loss (Hackney et al., 2008).

2.12 Conclusion

It is well established that unaccustomed eccentric exercise causes severe morphological changes to the musculoskeletal system. Whilst still debated, it is generally accepted that initial muscle damage occurs due to sarcomere disruption followed by E-C coupling failure. Increased intracellular Ca^{2+} concentrations then trigger proteolysis and an inflammatory response as the damaged muscle undergoes a period of degeneration and regeneration.

There is increasing evidence that endurance athletes are engaging in repeated bouts of resistance exercise to enhance endurance performance. Given the incidence of symptoms associated with EIMD following initial bouts of resistance exercise, the need to examine the effects of muscle-damaging exercise on endurance performance is paramount to endurance athletes and coaches. Therefore, the aim of this thesis was to investigate the implications of EIMD on the physiological, metabolic, perceptual and kinematic responses during endurance performance amongst recreationally active males.

CHAPTER 3

THE EFFECTS OF MUSCLE DAMAGING EXERCISE ON PHYSIOLOGICAL, METABOLIC, PERCEPTUAL AND KINEMATIC RESPONSES DURING TWO MODES OF ENDURANCE EXERCISE

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Burt, D.G., Twist, C., Lamb, K., & Nicholas, C. (2010). The effects of exercise-induced muscle damage on physiological and perceptual responses to cycling and running exercise. The Annual Conference of the British Association of Sport and Exercise Science, Glasgow, UK.

3.1 Introduction

A body of evidence has emerged that the development of neuromuscular characteristics from resistance-based exercise can further enhance endurance performance (Paavolainen et al., 1999; Paavolainen et al., 2000). Although the mechanisms are not fully understood, it is suggested that improvements in neuromuscular function translate into an improved exercise economy (Paavolainen et al., 1999). In particular, increased motor unit recruitment, improved muscle coordination, and enhanced utilisation of stored elastic energy following resistance-based exercise have been proposed as mechanisms which might reduce oxygen uptake ($\dot{V}O_2$) at a given exercise intensity (Johnston et al., 1997; Paavolainen et al., 1999; Hoff et al., 1999; Spurrs et al., 2003; Paton & Hopkins, 2005). However, while prolonged exposure to resistance training might improve performance in the long-term, a consequence of such training, particularly when it is unaccustomed, is the immediate and long-lasting appearance of symptoms associated with exercise-induced muscle damage (EIMD) in the days following (Byrne & Eston, 2002; Marginson et al., 2005; Twist & Eston, 2005). Such symptoms include increases in muscle soreness, swelling, elevated muscle proteins in the blood, impaired muscle function, and reduced neuromuscular control (Byrne et al., 2004). Furthermore, while maximal oxygen uptake appears to be unaltered after muscle-damaging exercise (Gleeson et al., 1998; Davies et al., 2011a), our understanding of the accompanying changes in oxidative metabolism is unclear. Some studies have observed no change in sub-maximal oxygen uptake after EIMD, suggesting that exercise economy remains stable (Gleeson et al., 1995; Walsh et al., 2001; Paschalis et al., 2005; Moysi et al., 2005; Marcora & Bosio, 2007; Davies et al., 2008; 2009; Twist & Eston, 2009), whereas others have observed increases in sub-maximal $\dot{V}O_2$ (Kyrolainen et

al., 2000; Calbert et al., 2001; Braun & Dutto, 2003; Chen et al., 2007b; Chen et al., 2008).

The challenge of interpreting these opposing results is made more difficult by the fact that studies have used different exercise protocols to induce muscle-damage and that the physiological responses after EIMD might be sensitive to the mode of endurance exercise adopted. Indeed, those studies showing no change in sub-maximal $\dot{V}O_2$ typically utilised cycling rather than running exercise. Therefore, it may be that alterations in limb kinematics (i.e. stride length and stride frequency) after EIMD have a greater impact on running than cycling. Several studies have shown that EIMD provokes changes to kinematic parameters during running (Hamill et al., 1991; Braun & Dutto, 2003; Paschalis et al., 2007; Chen et al., 2007b; 2009), and have associated these changes with increases in $\dot{V}O_2$ (Braun & Dutto, 2003; Chen et al., 2007b; 2009). The inference from such research is that muscle damage causes changes to a participant's stride pattern in order to limit the level of discomfort, and consequently an increase in oxygen cost occurs (Cavanagh & Williams, 1982). Given that stride length cannot deviate during cycling, it is unlikely that oxygen cost will increase after muscle damage due to such changes.

Alterations in lactate metabolism as a consequence of EIMD have not been reported consistently, with some studies reporting no change in [La] during sub-maximal exercise (Scott et al., 2003; Marcora & Bosio, 2007; Davies et al., 2008; 2011a; Twist & Eston, 2009) and others reporting a significant increase (Chen et al., 2007b; 2008; Schneider et al., 2007; Braun & Dutto, 2003; Gleeson et al., 1995). Elevated [La] responses might reflect an increased metabolic demand on undamaged fibres during exercise (Gleeson et al., 1998), or that damage occurs to type II muscle fibres

during eccentric exercise (Friden et al., 1983; Brockett et al., 2002) resulting in an increased activation of non-damaged type II fibres to enable the same level of force production. However, as is the case with the equivocal $\dot{V}O_2$ responses observed after muscle-damaging exercise, the different methods used to evoke symptoms of EIMD and the subsequent endurance exercise adopted make comparisons between such studies problematic.

Changes in the RPE during sub-maximal exercise suggest that participants demonstrate an altered sense of effort as a consequence of EIMD (Davies et al., 2008; Twist & Eston, 2009). Such responses may be associated with an increase in muscle pain after eccentric exercise, which has clearly been shown to heighten the sense of effort during force matching tasks (Proske et al., 2003; Weerakody et al., 2003). Similarly, increases in RPE have also coincided with an increased ventilatory response (Gleeson et al., 1995; Davies et al., 2008; Twist & Eston, 2009), which may transcend from a disruption to nerve afferents located in and around the blood vessels of exercising muscle that control ventilation (Haouzi et al., 1999; Haouzi et al., 2004; Hotta et al., 2006). It is known that ventilation and muscle pain are strong determinants of an individual's RPE response to exercise (Jameson & Ring, 2000; Hampson et al., 2001; Robertson, 1982), though, whether this is consistent for different endurance exercise modes is unknown.

Given the uncertainties highlighted above, this study aimed to 1) reaffirm that the muscle-damaging exercise (100 Smith-machine squats at 80% body mass) was effective in causing symptoms associated with EIMD; 2) to examine the effects of EIMD on endurance exercise, with a focus on whether $\dot{V}O_2$, \dot{V}_E , [La], RPE and kinematic responses are altered during endurance exercise as a result of EIMD; and

3) to ascertain whether the effects of EIMD on $\dot{V}O_2$, \dot{V}_E , [La], RPE and kinematic responses differ among contrasting modes of endurance exercise (namely cycling versus running). Findings from such a study would have implications for individuals contemplating concurrent resistance and endurance exercise for the first time. Indeed, if endurance exercise prescription is based on $\% \dot{V}O_{2peak}$, any increase in $\dot{V}O_2$ during endurance exercise as a result of EIMD will also affect training intensities in the ensuing days. For example, elevated $\dot{V}O_2$ in the days after EIMD would inform that individuals are exercising at a higher $\% \dot{V}O_{2peak}$ than they were prior to muscle damage.

3.2 Methods

3.2.1 Participants

Ten healthy male participants (age 22.8 ± 2.5 y, stature 1.77 ± 0.06 m, body mass 75.9 ± 8.8 kg), all of whom engaged in regular physical activity but had not undertaken any form of lower limb resistance exercise in the six months prior to assessment, volunteered to participate in the study. Prior to the study, each participant completed a written informed consent form and a health questionnaire and received a verbal explanation of the risks associated with the experimental procedures. Ethical approval was obtained from the Faculty of Health and Applied Sciences Research Ethics Committee, University of Chester.

3.2.2 Study design

Participants performed a cycling and a running incremental exhaustive test in a counter-balanced order, one week apart, to determine LT and peak oxygen uptake

($\dot{V}O_{2peak}$) for each exercise mode (see Table 1.). After the second incremental exhaustive test a habituation session was conducted to habituate participants with the procedures for measuring perceived muscle soreness and isokinetic muscle function. After a minimum of 48 h, participants returned to the laboratory to provide baseline measurements associated with two counter-balanced, 10 minute cycling and running exercise bouts (separated by 30 minutes of rest) at an exercise intensity corresponding to their individual LT (see Figure 3.1). After this and a further 15 minutes of passive recovery, participants completed a bout of lower limb resistance exercise designed to elicit muscle damage. They then returned 24 h and 48 h later to repeat the baseline procedures, in the same order. All participants were asked to refrain from any strenuous exercise 24 h before each test, maintain their normal diet, and avoid using any analgesic agents.

Table 3.1: Physiological measures (mean \pm SD) from two exhaustive exercise trials.

Variable	Exercise Mode	
	Cycling	Running
$\dot{V}O_{2peak}$ (ml·kg ⁻¹ ·min ⁻¹)	44.44 \pm 7.33	52.01 \pm 6.11
HR _{peak} (beats·min ⁻¹)	181 \pm 9	191 \pm 5
LT (mmol·l ⁻¹)	2.8 \pm 1.0	3.9 \pm 1.6
LT _{WR}	135.0 \pm 33.8 W	11.3 \pm 1.3 km·h ⁻¹
LT _{RPE}	13.4 \pm 1.1	13.6 \pm 1.0

Abbreviations: $\dot{V}O_{2peak}$ = peak oxygen uptake; HR_{peak} = peak heart rate at peak oxygen uptake; LT = lactate threshold; LT_{WR} = work rate corresponding to lactate threshold; LT_{RPE} = rating of perceived exertion at lactate threshold.

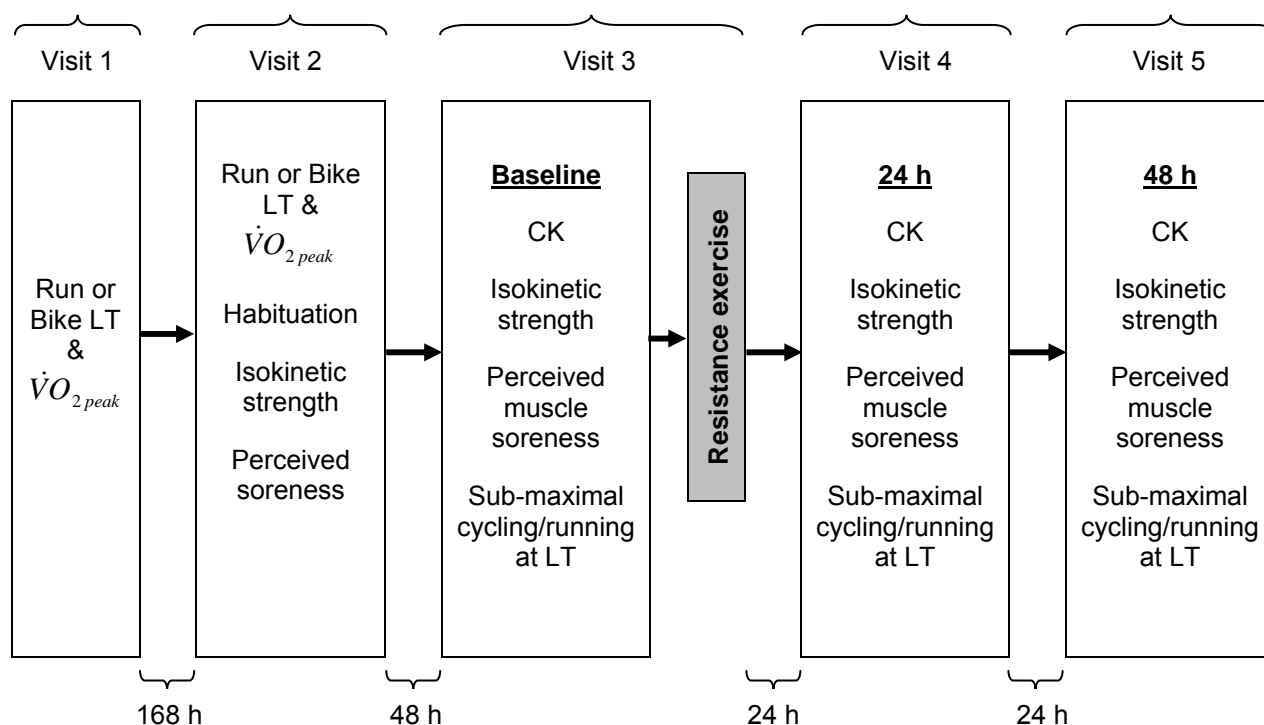


Figure 3.1 Schematic of the study design

3.3.3 Assessment of lactate threshold and peak oxygen uptake

Participants performed two incremental protocols to exhaustion using an electronically braked cycle ergometer (Lode Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands) and a motorised treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany), in order to determine their LT and $\dot{V}O_{2\text{ peak}}$ for both modes of exercise. The test commenced at a workload of 50 W (cycling) or a speed of 9 km·h⁻¹ with a 1% incline (running) and increased by 25 W or 0.5 km·h⁻¹ every 4 minutes until participants reached their LT. Individual LT was accepted as the power output or speed at which [La] increased 1 mmol·l⁻¹ above baseline values (Coyle et al., 1985). At this point, the resistance was increased by 25 W·min⁻¹ (cycling) and speed by 1 km·h⁻¹·min⁻¹ (running) until volitional exhaustion. Volitional exhaustion was defined as either the point at which participants could no longer maintain a cycling cadence between 60 – 80 rev·min⁻¹ or the speed of the treadmill.

Blood lactate levels were obtained from the Lactate Pro analyser (Arkray, Kyoto, Japan) using fingertip capillary blood samples taken during 30 s periods of forced inactivity between each 4 minute exercise bout.

Expired air was collected continuously throughout each exhaustive trial using an online metabolic system and calibrated prior to each test with a span gas mixture of 16% O_2 and 5% CO_2 and a 3 litre syringe (Hans Rudolph Inc., Kansas City, USA). Gas exchange variables, including oxygen uptake ($\dot{V}O_2$), were recorded breath-by-breath and later averaged over 30 s for each stage of the test. Heart rate (HR), collected via telemetry (Polar Electro, Polar Beat, Oy, Finland), was recorded in the final 15 s of each exercise bout. Upon exhaustion $\dot{V}O_{2\text{peak}}$ was accepted as the highest $\dot{V}O_2$ (averaged over 30 s). Reliability data for $\dot{V}O_{2\text{peak}}$ and HR at exhaustion showed a CV of 3.5% and 2.4%, respectively.

3.2.4 Assessment of rating of perceived exertion

Participants were asked to indicate their rating of perceived exertion using the Borg RPE scale. The scale ranges from 6 to 20, where 6 refers to 'no exertion at all' and 20 corresponds to 'maximal exertion'. During the final 15 s of the incremental tests to exhaustion and each sub-maximal exercise bout, participants were instructed to look at the scale and rate their overall perception of exertion (Borg, 1998). Before each maximal and sub-maximal exercise bout, participants were given detailed instructions on how to rate their effort using the scale (see Appendix 7). Reliability data for RPE at exhaustion and during sub-maximal exercise revealed a CV of 3.8% and 6.8%, respectively.

3.2.5 Assessment of peak isokinetic knee extensor torque

Isokinetic strength was measured using a Biodex dynamometer (Biodex, Multi-Joint System 3, Biodex Medical, NY, USA) at a velocity of $60 \text{ deg}\cdot\text{s}^{-1}$. Participants were sat upright with the knee and hip of the test limb fixed at 90° . The ankle was then fastened to the input arm of the dynamometer on the tibia just above the malleoli, with the rotational axis of the dynamometer aligned with the lateral femoral epicondyle ensuring only motion around the knee joint was possible. The upper body and opposite limb were secured with restraining straps to avoid any extraneous movement and the dynamometer input arm length, vertical and horizontal seat positions were recorded to ensure replication of testing positions for each visit. The total range of movement for each subject was manually determined, and the mass of the limb was recorded by the dynamometer to enable gravitational correction of peak torque values. After five sub-maximal and one maximal warm-up trials, participants performed five maximal efforts at $60 \text{ deg}\cdot\text{s}^{-1}$, from which the highest value (N·m) was recorded. Visual feedback, displaying real-time force, was used to encourage maximal efforts. Participants were also consistently encouraged to exceed target values, based on those achieved during the habituation and warm-up phases. Reliability data for peak isokinetic knee extensor torque at $60 \text{ deg}\cdot\text{s}^{-1}$ showed a coefficient of variation (CV) of 4.9%.

3.2.6 Perceived muscle soreness

With hands on hips and squatting to an approximate knee angle of 90° each participant was asked to provide his perceived level of muscle soreness of the knee extensors using a visual analogue scale (VAS; see Appendix 8 for an example). The VAS was numbered from 0-10 (on the reverse of the scale, unseen by the subject),

wherein 0 indicated no muscle soreness, 5 signified that the muscles felt sore upon movement and 10 that the muscles were too sore to move. This scale has been used successfully in previous research (Twist & Eston, 2009) and has been established as a valid and reliable measurement tool of soreness (Price et al., 1983).

3.2.7 Determination of creatine kinase

Plasma blood CK activity was assessed from a finger-tip capillary sample with the participant in a seated position. After pre-warming the hand via immersion in warm water (~42°C), a 30 microlitre sample of blood was collected, immediately pipetted to a test strip and analysed for CK using a colorimetric assay procedure (Reflotron, Boehringer Mannheim, Germany). Reliability data for CK revealed a CV of 10.9%.

3.2.8 Sub-maximal exercise protocols

The two 10 minute sub-maximal exercise protocols were performed in a counter-balanced order, separated by 30-min rest, on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) and a motorised treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany), at a workload and running speed corresponding to each participant's LT. Expired air, HR, RPE and [La] were recorded in the manner described above. Treadmill gradient was also set at a 1% incline to reflect the energetic cost of outdoor running (Jones & Doust, 1996). Reliability, assessed using CV, determined errors of 3.3%, 5.4%, 2.3% and 6% for $\dot{V}O_2$, \dot{V}_E , HR and [La], respectively.

3.2.9 Limb kinematics

Stride length and SF were determined by methods previously described by Braun and Dutto (2003) and Chen et al. (2007b, 2009). Briefly, a high speed video camera (Casio Exilim, Pro Ex-F1) recorded sagittal plane images at 100 Hz during the final 10 s of each 10-min sub-maximal running bout. The 10-s video recordings were then downloaded and digitized using motion analysis software (Quintec Biomechanics 9.03 v 14). Between 12 and 15 full strides were identified from the video recordings. SL for each full stride was then calculated from the formula: $SL\ (m) = velocity\ (m \cdot s^{-1}) / stride\ time\ (s)$, with an average SL used for analysis. SF was determined by measuring the time between the first and last heel contacts, and then dividing the number of full strides by time. For example, if 12 full strides were completed in 9.4 s, 12.8 strides would be completed in 10 s. Reliability data for SL and SF revealed a CV of 1.2% and 1% respectively.

3.2.10 Muscle-damaging exercise

To induce symptoms of muscle damage, participants performed an initial bout of 10 sets of 10 Smith-machine squats at 80% of body mass, with a 2 minute recovery between each set. Before starting the exercise, participants performed an unloaded squat during which a goniometer (Cranlea and Co., Birmingham, UK) was used to determine a 90 degree knee angle. Markers were then placed on either side of the Smith-machine to ensure the range of motion was maintained during the squatting protocol and between bouts. The bar was positioned on the participant's shoulders, with his back straight, legs fully extended, and feet hip-width apart. The eccentric phase of the squat involved lowering the bar until a knee angle of 90 degrees was achieved. Participants then lifted the bar back to the starting position to complete the

concentric phase. To stress the eccentric component, participants were instructed to ensure the downward and upward phases of the squat lasted for 2 and 1 s, respectively. Previous research has shown this protocol to be successful in inducing symptoms of muscle damage to the knee extensors (Byrne & Eston 2002).

3.2.11 Statistical analysis

Changes over time in the markers of muscle damage (CK, perceived muscle soreness and isokinetic strength), stride frequency and stride length were analysed using separate one-way repeated measures ANOVAs. To test for changes over time in the physiological, metabolic and perceptual variables between modes of exercise (cycling versus running) two-way (Time [3] x Mode [2]) repeated measures ANOVAs were applied. Assumptions of sphericity were assessed using Mauchly's test, with any violations adjusted by use of the Greenhouse-Geisser correction. *Post-hoc* Tukey tests, modified for repeated measures (Stevens, 2002), were used to determine where significant differences occurred. Descriptive statistics were calculated as means \pm SD. The alpha level was initially set at $P \leq 0.05$.

3.3 Results

3.3.1 Perceived muscle soreness

Perceived muscle soreness was found to change ($F_{(2,18)} = 114.2$, $P \leq 0.0005$) over time after muscle-damaging exercise (Figure 3.2), with values being significantly higher at 24 and 48 h post-baseline.

3.3.2 Plasma creatine kinase activity

Creatine kinase levels were found to vary over time after muscle-damaging exercise ($F_{GG(1.1, 9.9)} = 7.2$, $P = 0.021$). Baseline values ($78.1 \pm 30.4 \text{ U}\cdot\text{l}^{-1}$) were significantly higher at 24 h ($238.9 \pm 181.6 \text{ U}\cdot\text{l}^{-1}$) but not at 48 h ($143.1 \pm 83.9 \text{ U}\cdot\text{l}^{-1}$).

3.3.3 Peak knee extensor torque

Peak knee extensor torque decreased after muscle-damaging exercise ($F_{GG(1.0, 9.3)} = 10.1$, $P = 0.01$), with values at 24 and 48 h being significantly lower than baseline (Figure 3.3).

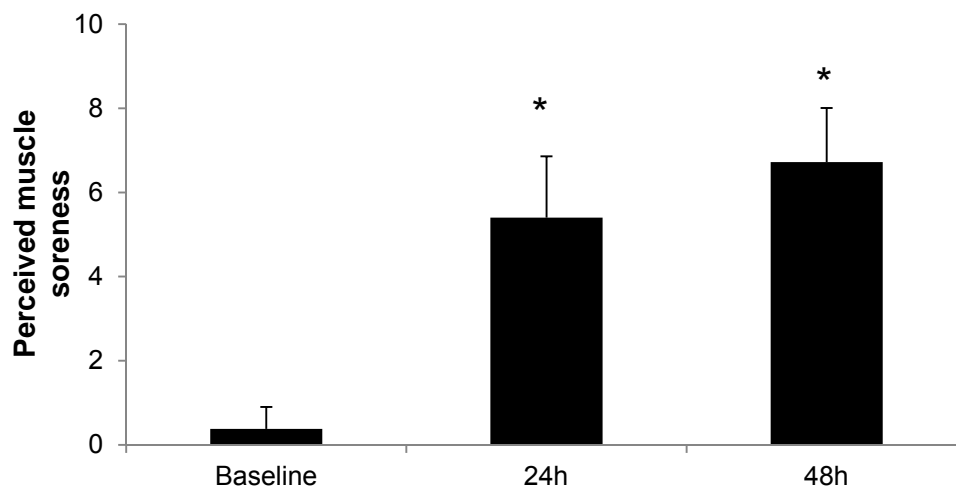


Figure 3.2: Changes in perceived muscle soreness (means \pm SD) after 100 squats. * Significantly different to baseline ($P < 0.05$).

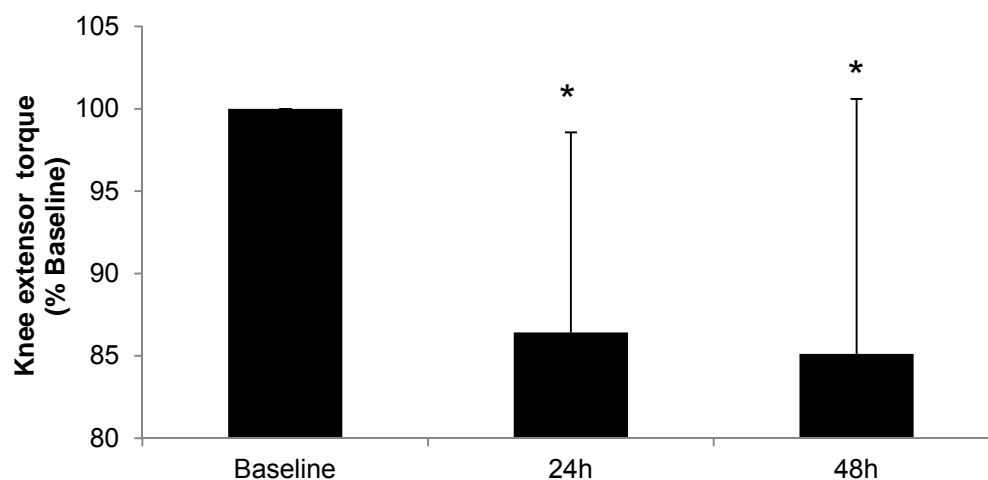


Figure 3.3: Changes in relative peak isokinetic knee extensor torque (means \pm SD) after 100 squats. * Significantly different to baseline ($P < 0.05$).

3.3.4 Sub-maximal cycling responses to muscle-damaging exercise

There was a significant main effect of Time on $\dot{V}O_2$ ($F_{2,18} = 4.6$, $P = 0.025$), \dot{V}_E ($F_{2,18} = 6.5$, $P = 0.008$), $\dot{V}_E / \dot{V}O_2$ ($F_{2,18} = 6.5$, $P = 0.008$), $\dot{V}_E / \dot{V}CO_2$ ($F_{2,18} = 12.3$, $P = 0.0001$), f_R ($F_{(GG)1.24, 11.13} = 13.5$, $P = 0.003$), HR ($F_{2,18} = 6.1$, $P = 0.010$) and RPE ($F_{2,18} = 15.2$, $P = 0.0001$) after muscle-damaging exercise. Post-hoc analysis revealed that $\dot{V}O_2$, \dot{V}_E , $\dot{V}_E / \dot{V}O_2$, $\dot{V}_E / \dot{V}CO_2$, f_R and HR values were significantly higher at 48 h than baseline, whilst RPE was significantly increased above baseline at both 24 h and 48 h ($P < 0.05$). However, there was no significant Time effect on $\dot{V}CO_2$ ($F_{(GG)1.3, 11.7} = 2.0$, $P = 0.186$), [La] response ($F_{2,18} = 1.3$, $P = 0.292$) or mean RPM ($F_{2,18} = 1.4$, $P = 0.274$) (Table 3.2).

3.3.5 Sub-maximal running responses to muscle-damaging exercise

There was a significant main effect of Time on $\dot{V}O_2$ ($F_{2,18} = 6.4$, $P = 0.008$), \dot{V}_E ($F_{2,18} = 12.7$, $P = 0.0001$), $\dot{V}_E / \dot{V}CO_2$ ($F_{2,18} = 11.4$, $P = 0.001$), f_R ($F_{2,18} = 7.3$, $P = 0.005$) and RPE ($F_{2,18} = 11.9$, $P = 0.001$), with values being significantly higher than baseline at 24 h and 48 h. $\dot{V}_E / \dot{V}O_2$ ($F_{(GG) 1.2, 10.8} = 8.7$, $P = 0.011$) was also increased after muscle damage, although values were only significant at 24 h. The main effect of Time on $\dot{V}CO_2$ ($F_{2,18} = 3.2$, $P = 0.066$), HR ($F_{2,18} = 2.1$, $P = 0.154$), or [La] response ($F_{2,18} = 2.1$, $P = 0.148$) was not significant. These changes were accompanied by significant decreases in stride length ($F_{(GG) 1.07, 9.7} = 9.2$, $P = 0.012$) and increases in stride frequency ($F_{(GG) 1.1, 9.9} = 6.98$, $P = 0.023$) at 24 and 48 h after the squatting exercise. Values for running are shown in Table 3.2.

3.3.6 Sub-maximal responses to muscle-damaging exercise – cycling versus running

There was no significant interaction of Time x Mode on $\dot{V}O_2$ ($F_{2,18} = 1.3$, $P = 0.308$), $\dot{V}CO_2$ ($F_{2,18} = 2.0$, $P = 0.167$), f_R ($F_{(GG)1.1, 10.1} = 4.5$, $P = 0.057$), HR ($F_{2,18} = 1.4$, $P = 0.280$), RPE ($F_{2,18} = 0.9$, $P = 0.414$) or [La] response ($F_{2,18} = 2.5$, $P = 0.108$) after muscle-damaging exercise. However, there was a significant Time x Mode interaction on \dot{V}_E ($F_{2,18} = 5.3$, $P = 0.016$), which reflected a higher relative increase for running at 24 h post EIMD than cycling. Similarly, $\dot{V}_E / \dot{V}O_2$ ($F_{2,18} = 6.2$, $P = 0.009$) and $\dot{V}_E / \dot{V}CO_2$ ($F_{2,18} = 7.0$, $P = 0.006$) were also shown to be higher at 24 h during running when compared to cycling (Table 3.3).

Table 3.2: Mean (\pm SD) physiological, metabolic, perceptual and kinematic responses to sub-maximal exercise at LT after 100 squats.

	Cycling			Running		
	Baseline	24 h	48 h	Baseline	24 h	48 h
$\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	28.08 \pm 5.58	28.81 \pm 6.02	29.67 \pm 6.53*	41.59 \pm 4.82	43.01 \pm 4.89*	43.15 \pm 5.40*
$\dot{V}CO_2$ (ml·kg ⁻¹ ·min ⁻¹)	26.91 \pm 4.60	26.99 \pm 5.66	28.12 \pm 6.16	40.38 \pm 4.51	41.90 \pm 4.95	41.99 \pm 5.47
\dot{V}_E (l·min ⁻¹)	58.4 \pm 10.18	60.9 \pm 15.13	66.2 \pm 16.94*	89.20 \pm 11.35	99.30 \pm 14.84*	97.20 \pm 14.79*
$\dot{V}_E / \dot{V}O_2$	27.72 \pm 2.49	27.89 \pm 2.98	29.45 \pm 2.81*	28.52 \pm 3.4	30.64 \pm 4.06*	29.92 \pm 4.41
$\dot{V}_E / \dot{V}CO_2$	28.81 \pm 2.17	29.79 \pm 2.48*	30.99 \pm 1.73*	29.42 \pm 3.00	31.60 \pm 3.70*	30.76 \pm 3.38
RPE	13.5 \pm 1.4	15.2 \pm 1.6*	15.4 \pm 1.8*	14.7 \pm 0.8	16.1 \pm 1.8*	16.5 \pm 1.9*
f_R (min ⁻¹)	27.9 \pm 5.2	30.6 \pm 5.95	35.3 \pm 6.33*	40.1 \pm 8.57	44.8 \pm 9.0*	43.3 \pm 10.2
HR (b·min ⁻¹)	132.9 \pm 16.7	137.4 \pm 15.0	140.4 \pm 17.9*	162.2 \pm 14.3	167.2 \pm 10.9	167.2 \pm 11.0
[La] (mmol·l ⁻¹)	3.48 \pm 1.53	3.40 \pm 1.57	3.69 \pm 1.58	4.08 \pm 1.91	4.54 \pm 2.21	4.43 \pm 2.16
RPM	79.8 \pm 15.7	75.6 \pm 18.3	77.8 \pm 18.4	-	-	-
SL (m)	-	-	-	2.24 \pm 0.26	2.20 \pm 0.28*	2.20 \pm 0.27*
SF	-	-	-	14.1 \pm 0.6	14.4 \pm 0.8*	14.4 \pm 0.8*

* Significantly different to baseline ($P < 0.05$). *Abbreviations:* $\dot{V}O_2$ = volume of oxygen uptake, $\dot{V}CO_2$ = volume of carbon dioxide, \dot{V}_E = minute ventilation, $\dot{V}_E / \dot{V}O_2$ = ventilatory equivalent for oxygen, $\dot{V}_E / \dot{V}CO_2$ = ventilatory equivalent for carbon dioxide, RPE = rating of perceived exertion, f_R = breathing frequency, HR = heart rate, [La] = blood lactate, RPM = revolutions per minute, SL = stride length, SF = stride frequency.

Table 3.3: Normalised (relative to baseline) changes to physiological, metabolic, and perceptual responses to sub-maximal cycling and running after 100 squats. Values are expressed as means \pm SD.

	Cycling		Running	
	24 h	48 h	24 h	48 h
$\dot{V}O_2$	+2.4 \pm 6.7%	+5.3 \pm 6.6%	+3.5 \pm 4.6%	+3.7 \pm 3.1%
$\dot{V}CO_2$	-0.2 \pm 9.1%	+3.8 \pm 9.5%	+3.8 \pm 5.2%	+3.9 \pm 5.9%
\dot{V}_E^*	+3.4 \pm 11.6%	+12.3 \pm 13.1%	+11.3 \pm 8.3%	+9.0 \pm 8.4%
$\dot{V}_{E/I}\dot{V}O_2^*$	+0.6 \pm 5.7%	+6.5 \pm 7.8%	+7.5 \pm 6.6%	+4.8 \pm 6.7%
$\dot{V}_{E/I}\dot{V}CO_2^*$	+3.4 \pm 3.5%	+7.9 \pm 6.3%	+7.4 \pm 5.5%	+4.7 \pm 6.0%
RPE	+12.7 \pm 7.2%	+14.3 \pm 10.8%	+9.3 \pm 8.0%	+12.1 \pm 9.5%
f_R	+11.2 \pm 24.3%	+28.5 \pm 26.4%	+12.4 \pm 10.7%	+8.2 \pm 13.2%
HR	+3.7 \pm 5.0%	+5.7 \pm 5.7%	+3.4 \pm 5.8%	+3.5 \pm 7.4%
[La]	-2.4 \pm 20.3%	+6.4 \pm 15.0%	+10.2 \pm 20.2%	+9.1 \pm 24.5%

* Significant difference between cycling and running at 24 h ($P < 0.05$)

3.4 Discussion

The changes observed in muscle function, perceived muscle soreness and plasma CK indicate that the squatting protocol used in this study was effective in inducing symptoms associated with EIMD. Specifically, isokinetic peak knee extensor torque significantly decreased by 13.6% and 14.9% from baseline values at 24 and 48 h, respectively. This magnitude of force loss concurs with previous studies that have adopted resistance exercise (Byrne & Eston, 2002a; Davies et al., 2008; 2009) plyometrics (Twist & Eston, 2009) and downhill running (Braun & Dutto, 2003) for inducing muscle damage. In addition, the elevated levels of perceived muscle soreness (at both 24 and 48 h) are findings consistent with those reported studies that adopted a similar method of muscle-damaging exercise (Davies et al., 2008; 2009). Likewise, the observed CK response was similar to that reported by Byrne and Eston (2002a), but whilst it was elevated above baseline, its peak occurred at 24 h and not 48 h (as with muscle function and perceived muscle soreness). This pattern of response has been observed with previous research and might reflect an accelerated clearance of CK from the blood after 24 h (Hyatt & Clarkson, 1998; Warren et al., 1999).

It is notable that the physiological and perceptual responses to sub-maximal cycling and running exercise in the current study were altered as a result of muscle damage. In terms of $\dot{V}O_2$, increases occurred during both modes of exercise, though the time course appeared to be different. That is, the $\dot{V}O_2$ response during running was significantly elevated above pre-EIMD values from 24 – 48 h, and concurs with previous findings (Kyrolainen et al., 2000; Calbet et al., 2001; Braun & Dutto, 2003; Chen et al., 2007b; 2009), whereas the cycling $\dot{V}O_2$ response was only significantly

higher after 48 h. This is a novel and unexpected finding in light of past research (Gleeson et al., 1995; Walsh et al., 2001; Moysi et al., 2005; Davies et al., 2009; Twist & Eston, 2009) and deserves further scrutiny.

Cavanagh and Williams (1982) reported an increased $\dot{V}O_2$ response when runners deviated from their optimal stride length. Therefore, it is feasible that the elevated $\dot{V}O_2$ response during sub-maximal running was due in part to changes in lower limb kinematics following muscle-damaging exercise. Indeed, that this increased oxygen cost of running coincided with alterations in stride length and frequency, and that changes in $\dot{V}O_2$ during sub-maximal running were found to be inversely correlated with changes in stride length after EIMD for 7 of the 10 participants ($r = -0.71 - -0.92$), reinforces the findings of several running-related studies (Braun & Dutto, 2003; Paschalis et al., 2007; Chen et al., 2007b; 2009). Moreover, that participants experienced heightened muscle soreness 24 and 48 h after the squatting exercise might explain the shortened stride length as a strategy to limit their discomfort. However, whilst kinematic changes could have influenced the time course response of running $\dot{V}O_2$ after EIMD, the observation of Hamill et al. (1991) that minor kinematic changes occurred without any concomitant rise in oxygen cost imply that other mechanisms were involved.

Chen et al. (2007b; 2009) attributed post-EIMD decrements in running economy between 24 – 72 h to an impaired ability to utilise the SSC. Previous research has demonstrated that improvements in running economy are a result of increased musculotendinous stiffness, allowing the muscle to absorb and utilise elastic energy more effectively during the SSC (Spurrs et al., 2003). However, EIMD has been shown to reduce stretch reflex sensitivity and muscle stiffness regulation, leading to

a decline in the force potential of the SSC (Nicol et al., 1996; Horita et al., 1996; 1999; Avela & Komi, 1998; Byrne & Eston, 2002a). Therefore, it is possible that reductions in musculotendinous stiffness after squatting exercise could have occurred as early as 24 h leading to a decreased ability to absorb and utilise elastic energy during running, creating an increased energy cost. Although, as such changes have yet to be demonstrated empirically during running, this is an area for future investigation.

The aforementioned post-EIMD increase in $\dot{V}O_2$ during sub-maximal cycling is at odds with previous research. Adopting a similar mode of muscle-damaging exercise, with comparable decrements in muscle function, both Moysi et al. (2005) and Davies et al. (2009) reported no changes in $\dot{V}O_2$ during sub-maximal cycling. Studies using plyometrics (Twist & Eston, 2009) and eccentric bench stepping (Gleeson et al., 1995; Schneider et al., 2007) to induce muscle damage also reported unchanged $\dot{V}O_2$ responses during sub-maximal cycling. Greater physiological responses have been reported during cycling at slower pedalling cadences (Deschenes et al., 2000). However, changes in cycling cadence are unable to explain the increase in $\dot{V}O_2$ as RPM remained unchanged (see Table 3.2). The elevation in $\dot{V}O_2$ might have been due to the activation of auxiliary muscles after muscle-damaging exercise. For example, knee extensor strength had decreased by approximately 15% 48 h after muscle damage, suggesting that increased motor unit activation may have been evident in order to generate the same level of force required during the fixed-intensity cycling (Kyrolainen et al., 2000; Braun & Dutto, 2003; Elmer et al., 2010). Indeed, as Bigland-Ritchie and Woods (1974) have observed a linear relationship exists between EMG activity and oxygen cost, it is possible that elevations in $\dot{V}O_2$

during cycling might be explained by increased recruitment of motor units after EIMD. This requires further investigation. The only other study to observe an increase in $\dot{V}O_2$ during sub-maximal cycling after muscle-damaging exercise postulated that it might have been due to an increase in ventilation (Burt & Twist, 2011). As the current elevations in $\dot{V}O_2$ during both cycling and running occurred alongside increases in \dot{V}_E (see Table 3.2 and 3.3), it is possible that the lungs evoked an added metabolic cost in order to facilitate the augmented \dot{V}_E response.

The elevated \dot{V}_E responses concur with previous studies during both sub-maximal running (Braun & Dutto, 2003; Chen et al., 2007b; 2008; 2009) and cycling exercise (Gleeson et al., 2005; Schneider et al., 2007; Davies et al., 2008; 2009; Twist & Eston, 2009). As with the $\dot{V}O_2$ response, the differing time course of \dot{V}_E and f_R during running and cycling after EIMD was not hypothesised and is difficult to explain. However, the following are offered as possible contributing factors. In contrast to cycling, running utilises the SSC, and there is strong evidence that the stretch-reflex has a vital role in SSC activity and contributes to force generation between eccentric and concentric phases (Komi, 2000). The reduction in stretch-reflex sensitivity after muscle damage is thought to be mediated by the activation of fine myelinated (Group III) and unmyelinated (Group IV) afferent fibres (Horita et al., 1996; 1999; Nicol et al., 1996; Avela & Komi, 1998; Avela et al., 1999). These small afferents could also exert a significant influence on \dot{V}_E during dynamic exercise (Matieka & Duffin, 1995; Davies et al., 2011a). Therefore, it is possible that activation of Group III and IV afferents inhibiting stretch-reflex sensitivity as a consequence of EIMD may have led to the early augmentation of \dot{V}_E during running. Group III and IV afferent fibres are often described as being polymodal, in that they are sensitive to

several parameters associated with fatigue or muscle damage (Avela & Komi, 1998; Haouzi et al., 2004). Muscle pain has also been shown to provoke an increased ventilatory response through activating nociceptive muscle afferents (Mense, 1977; 1982; Duranti et al., 1991). Therefore, such pain (soreness) after the squatting exercise may have also stimulated afferent fibres contributing to the increased ventilatory response during running and cycling. Furthermore, the heightened muscle soreness response after 48 h could have been responsible for the increase in \dot{V}_E and f_R during cycling after 48 h.

Alternatively, an increased ventilatory response whilst exercising with muscle damage has been attributed to alterations in metabolic pathways (Gleeson et al., 1995). Since [La] values in the current study were not statistically different during either cycling or running after muscle damage, the increase in \dot{V}_E cannot be attributed to an increase respiratory response to cope with increased acidosis. Furthermore, post-EIMD $\dot{V}_E/\dot{V}CO_2$ was also elevated during both modes of exercise, indicating that \dot{V}_E was not increased to excrete accumulating CO_2 after squatting exercise (Twist & Eston, 2009).

Although there was no difference in RPE response, both modes of fixed-intensity exercise revealed significant increases in effort perception 24 h and 48 h after EIMD. These results are consistent with previous research during cycling (Davies et al., 2009; Twist & Eston, 2009) and running (Scott et al., 2003; Chen et al., 2009) as a result of exercise-induced muscle damage. Jameson and Ring (2000) suggested that effort perception during endurance exercise is based on a combination of both peripheral (increased leg pain) and central (feelings of breathlessness) feedback. The increased knee extensor soreness reported in the current study could have

provided the peripheral cue, whilst, the elevated \dot{V}_E and f_R responses reported during both modes of exercise provided the central cue (Davies et al., 2009). Moreover, activation of mechanoreceptors in the chest wall, lungs and airways may have increased breathing rate, influencing the participant in perceiving exercise to be harder after squatting exercise (Hampson et al., 2001). Marcora (2009) challenged the view that effort perception during exercise is dependent on feedback from the skeletal muscle, heart and lungs, suggesting that an increase in RPE during exercise is centrally governed from the brain. However, Amann et al. (2010) reported that through blocking afferent feedback response from the locomotor muscles a reduction in \dot{V}_E and RPE responses occurred. Arguably, therefore, muscle afferents in the current study may have influenced RPE responses during both cycling and running modes after muscle-damaging exercise.

Although attempts were made to ensure that the exercise intensities between running and cycling were similar, it is possible that our findings were influenced by the participants' training history. Whilst, they were engaged in regular endurance exercise (2-3 sessions per week), this mainly consisted of running-based activity with limited cycling training. Unsurprisingly, symptoms associated with EIMD are dependent on training history, with greater responses reported in less active muscle groups compared to those regularly exposed to exercise (Chen et al., 2011; Jamurtas et al., 2005). Furthermore, individuals more accustomed with running are able to exercise at a higher intensity for a given RPE compared to cycling (Thomas et al., 1995). Therefore, it is possible that participants in the current study tolerated running with muscle damage due to their greater familiarity with the exercise modality, whilst the unexpected increase in $\dot{V}O_2$ during cycling might have occurred due to participants being less familiar with this type of exercise.

In conclusion, the equivocal findings of EIMD on endurance performance have been attributed to the mode of endurance exercise adopted. The aim of Study 1 was to examine the influence of exercise mode (cycling versus running) on physiological, metabolic, perceptual and kinematic responses after EIMD. To address this aim, participants performed two counter-balanced cycling and running bouts before, 24 h and 48 h after muscle-damaging exercise. Findings demonstrated that $\dot{V}O_2$ and \dot{V}_E were increased during both running and cycling modes after EIMD. However, the time course of these appeared to be mode-specific. It is posited that the elevated $\dot{V}O_2$ responses observed during running were due to changes in lower limb kinematics and a decreased ability to utilise the SSC, whilst the recruitment of auxiliary muscles after EIMD might have led to the unexpected increase in cycling $\dot{V}O_2$ response. The differences in ventilatory response between exercise modes following muscle damage were possibly due to different stimuli activating afferent muscle fibres. This is the first study to investigate the effects of EIMD during sub-maximal cycling and running exercise. From an applied perspective, given that long-term resistance exercise can improve endurance performance, individuals considering concurrent training should be aware of the consequences that unaccustomed resistance exercise can have on sub-maximal endurance exercise performed in the days following. Whilst, this study reaffirms that EIMD increases oxygen uptake during endurance exercise, a progression for the next study will be to consider how muscle damage affects oxidative metabolism at rest and during recovery from endurance exercise.

CHAPTER 4

**THE EFFECTS OF EXERCISE-INDUCED MUSCLE DAMAGE ON RESTING
METABOLIC RATE, SUB-MAXIMAL RUNNING AND POST-EXERCISE OXYGEN
CONSUMPTION**

The contents of this chapter have previously been published in the following:

Burt, D.G., Lamb, K., Nicholas, C., & Twist, C. (2013). The effects of exercise-induced muscle damage on resting metabolic rate, sub-maximal running and post-exercise oxygen consumption. *European Journal of Sport Science*, DOI:10.1080/17461391.2013.783628

4.1 Introduction

It is now established that regular resistance exercise improves several key parameters of endurance performance, including running economy (RE) (Johnston et al., 1997; Paavolainen et al., 1999; Millet et al., 2002; Storen et al., 2008). Resistance exercise can also facilitate increases in resting metabolic rate (RMR) (Pratley et al., 1994; Dolezal & Potteiger, 1998; Paschalis et al., 2011). Indeed, several studies have observed increases in RMR for up to 72 hours after an acute bout of resistance exercise (Williamson & Kirwan, 1997; Dolezal et al., 2000; Schuenke et al., 2002; Jamurtas et al., 2004; Hackney et al., 2008; Paschalis et al., 2010; 2011). Typically, these studies used resistance exercises incorporating a high eccentric component, in which the muscle lengthens under tension and yields a force greater than that of concentric actions despite fewer motor units being recruited (Enoka, 1996). However, such exercise places substantial stress on the muscle, and when executed during an unaccustomed activity can lead to the immediate and prolonged appearance of symptoms associated with EIMD (Byrne et al., 2004).

The initial manifestations of EIMD include a disruption to the sarcomeres and damage to the excitation-contraction (E-C) coupling system. This is followed by an inflammatory response, which promotes the breakdown, removal and resynthesis of the damaged muscle fibre (Armstrong et al., 1991; Morgan & Allen, 1999; Proske & Morgan, 2001; Byrne et al., 2004). Furthermore, the inflammatory response associated with EIMD is responsible for the elevation in RMR after acute eccentric exercise (Dolezal et al., 2000; Schuenke et al., 2002; Jamurtas et al., 2004; Paschalis et al., 2010; 2011). The repair of damaged muscle is reported to be energetically expensive, with the energy cost of protein resynthesis estimated to account for as much as 20% of the RMR (Welle & Nair, 1990).

Symptoms of EIMD include delayed onset muscle soreness (DOMS), leakage of intramuscular proteins (e.g. creatine kinase) into circulation and the impairment of muscle function (Byrne et al., 2004). Furthermore, at their peak, typically 24 – 48 h after damage-inducing exercise, these symptoms are detrimental to sub-maximal endurance performance. Indeed, as observed in Chapter 3, alterations in oxygen cost, minute ventilation, blood lactate, ratings of perceived exertion and lower limb kinematics occurred during sub-maximal running as a result of EIMD.

Oxygen uptake does not return from its elevated state to resting values immediately after the termination of endurance exercise. Traditionally, this post-exercise increased oxygen consumption has been attributed to an oxygen debt, which was deemed necessary to repay the oxygen deficiency that occurred at the onset of exercise (Gaesser & Brooks, 1984; Bahr, 1992; Borsheim & Bahr, 2003). Hill and Lupton's (1923) original hypothesis that the oxygen debt resulted from the oxidation of lactate was developed by Margaria et al. (1933). The authors posited that the oxygen debt comprised both an alactacid component, which was responsible for the restoration of phosphagens, and a lactacid component responsible for the resynthesis of glycogen from lactate (Margaria et al., 1933). More recent studies have challenged this hypothesis, observing that the magnitude of the oxygen debt is neither equal to nor correlates with the oxygen deficit (Gaesser & Brooks, 1984; Bahr, 1992). Therefore, it was suggested that the term 'excess post-exercise oxygen consumption (EPOC)' was more appropriate for the prolonged increase in oxygen cost that can be evident for several hours after the cessation of exercise (Gaesser & Brooks, 1984; Brehm, 1988; Bahr, 1992; Borsheim & Bahr, 2003; Laforgia et al., 2006).

The magnitude of EPOC is often dependent upon the intensity of the exercise adopted (Sedlock et al., 1989; Bahr & Sejersted, 1991; Bahr, 1992; Smith & McNaughton, 1993). Therefore, increases in oxygen cost during fixed intensity endurance exercise when muscle is damaged (as shown in Chapter 3) could elevate EPOC further. Indeed, when examining the impact of strenuous training on recovery from prior strenuous exercise, Bahr et al. (1991) reported that the increase in oxygen demand during sub-maximal cycling exercise was responsible for a 24% increase in oxygen uptake during recovery post-exercise. However, to what extent muscle damage from prior resistance exercise alters EPOC after sub-maximal endurance exercise is currently not known. Such knowledge might have implications for endurance athletes who engage in repeated bouts of endurance exercise on the same day or in the days after. Indeed, if $\dot{V}O_2$ is still elevated after exercising with muscle damage, reductions in exercise efficiency during subsequent endurance training sessions are likely to occur (Bahr et al., 1991; Borsheim & Bahr, 2003). Furthermore, given that findings from previous research and Chapter 3 demonstrate that EIMD increases oxygen uptake at rest and during endurance exercise, it would be interesting to examine whether oxygen uptake during recovery from endurance exercise is increased when experiencing EIMD. Thus, this investigation aimed to 1) confirm that resting metabolic rate is elevated for up to 48 h after muscle-damaging exercise; 2) reaffirm that oxygen uptake during sub-maximal running exercise is increased for up to 48 h after EIMD; and 3) to examine whether elevations in oxidative metabolism at rest and during endurance exercise after EIMD increase post-exercise oxygen consumption.

4.2 Methods

4.2.1 Participants

Eight healthy male participants (age 24.3 ± 1.7 y, stature 1.81 ± 0.08 m, body mass 77.4 ± 10.8 kg), all of whom engaged in regular physical activity (2 – 3 sessions per week) but had not undertaken any form of lower limb resistance exercise in the six months prior to assessment, volunteered to take part in the study. Participants' physiological characteristics are shown in Table 1. Each participant completed a written informed consent form and a health questionnaire and received a verbal explanation of the risks associated with the experimental procedures. Ethical approval was obtained from the Faculty of Applied Sciences Research Ethics Committee, University of Chester.

4.2.2 Study Design

This was a within-subjects design involving multiple repeat measurements interspersed with an intervention to invoke symptoms of EIMD (see Figure 1.). Participants completed a habituation session to accustomise them with the procedures used for measuring resting metabolic rate (RMR), perceived muscle soreness and isokinetic strength. During the same visit they also completed an incremental running trial to determine individual lactate turnpoint (LTP) and peak oxygen uptake ($\dot{V}O_{2peak}$). One week later, participants completed baseline measurements, which included 45 minutes of supine rest to measure RMR, three indirect markers of EIMD, 10 minutes of sub-maximal running, followed by a 30-minute recovery period to assess EPOC. Twenty four hours after this, they completed a bout of resistance exercise designed to cause symptoms of EIMD. They then returned to the laboratory 24 and 48 h later to repeat the baseline procedures

(in the same order). All measurements were conducted between 08:00 and 09:00 h after a 12 h overnight fast. Participants were asked to refrain from any other exercise 24 h before each trial, maintain a similar diet for each testing day and avoid using any analgesic agents.

Table 4.1. Physiological characteristics

Variable	Mean \pm SD
$\dot{V}O_{2\text{peak}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	54.2 ± 5.5
HR_{peak} ($\text{b}\cdot\text{min}^{-1}$)	189 ± 5
LTP ($\text{mmol}\cdot\text{l}^{-1}$)	4.4 ± 0.8
$\text{LTP}_{\text{SPEED}}$ ($\text{km}\cdot\text{h}^{-1}$)	11.9 ± 1.0

Abbreviations: $\dot{V}O_{2\text{peak}}$ = peak oxygen uptake; HR_{peak} = peak heart rate at peak oxygen uptake; LTP = lactate turnpoint; $\text{LTP}_{\text{SPEED}}$ = speed corresponding to lactate turnpoint.

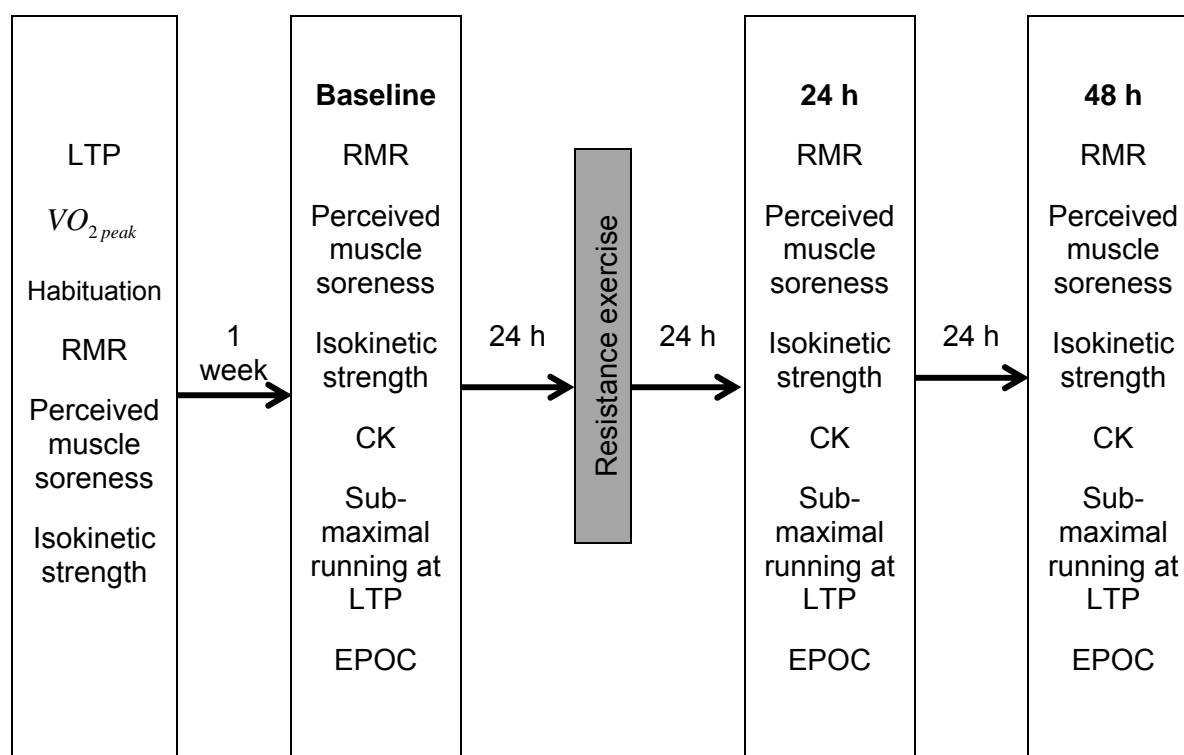


Figure 4.1. Schematic of the study design

4.2.3 Assessment of lactate turnpoint and peak oxygen uptake

Participants performed an incremental treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany) protocol to determine individual LTP and $\dot{V}O_{2peak}$. The protocol started at 9 km·h⁻¹ and increased by 1.0 km·h⁻¹ every 4 minutes until volitional exhaustion. Blood lactate activity was obtained via the Lactate Pro analyser (Arkray, Kyoto, Japan) from finger-tip capillary blood samples taken during 30 s periods of forced inactivity between each 4-minute exercise bout. Individual LTP was accepted as the speed at which a second distinctive rise in [La] occurred above baseline values (Jones et al., 2009). Expired air, HR and RPE were recorded and analysed in the manner described in Chapter 3.

4.2.4 Perceived muscle soreness

Perceived muscle soreness in the knee extensors was measured using the same scale described in Chapter 3.

4.2.5 Assessment of dynamic muscle function

Knee extensor strength of the dominant limb was measured using the same procedures outlined in Chapter 3.

4.2.6 Determination of creatine kinase

Creatine kinase activity was assessed from the same methods used in Chapter 3.

4.2.7 Assessment of resting metabolic rate

Resting metabolic rate was measured whilst participants adopted a supine position on a bed in a dark, quiet and thermo-controlled laboratory (22 ± 0.4 °C) for 45

minutes. Expired air was collected using an online gas analyser (Oxycon Pro, Hoechberg, Germany), calibrated before each trial in the manner highlighted in Chapter 3. Heart rate (Polar Electro, Polar Beat, Oy, Finland) was also recorded during the RMR collection period. Data during the first 15 minutes of measurement were discarded and the average of the last 30 minutes was used to determine RMR (Osterberg & Melby, 2000). Participants were instructed to remain quiet, minimise their movements and avoid hyperventilation and sleep during the RMR collection period. Prior to each RMR trial they were requested not to eat or drink, except water, for 12 h beforehand and to refrain from exercise (additional to that provided in the study) for 48 h. Energy expenditure (kcal) was calculated using the Weir equation (1949) and expressed per 24 h (Paschalis et al., 2010).

4.2.8 Assessment of energy intake

Participants were provided with written guidelines and a food diary to record their daily food intake. They were instructed to monitor and follow their normal dietary habits 24 h before the first visit and asked to maintain those foods in similar amounts until the last day of their involvement in the study (Dolezal et al., 2000).

4.2.9 Sub-maximal running protocol

Participants were required to run on a motorised treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany) at a speed corresponding to their previously determined LTP for 10 minutes. Expired air, HR, RPR and [La] were recored in the final minute of each bout using methods described in Chapter 3.

4.2.10 Assessment of post-exercise oxygen consumption

After each sub-maximal running bout, participants resumed the supine resting position and their expired air and HR were recorded immediately for 30 minutes. EPOC was determined in the manner described by Binzen et al. (2001):

$$\text{EPOC (l)} = \text{total recovery } \dot{V}O_2 - \text{total baseline RMR } \dot{V}O_2.$$

A recovery period of 30 minutes was based on studies (incorporating exercise of similar magnitude and intensity to that used in the current study), in which EPOC had been observed to return to baseline within 30 minutes (Freedman-Akabas et al., 1985; Lee et al., 1999).

4.2.11 Muscle-damaging exercise

Participants performed the same muscle-damaging exercise as described in Chapter 3.

4.2.12 Statistical analysis

The variability of the dependent variables (indirect markers of muscle-damage, RMR, sub-maximal running and EPOC) across the three trials (Time) was examined via separate one-way repeated measures analysis of variance (ANOVAs). Assumptions of sphericity were assessed using Mauchly's test and any violations were adjusted by Greenhouse-Geisser correction. *Post hoc* Tukey tests, modified for repeated measures (Stevens, 2002), were applied where appropriate to determine where significant differences in means occurred. Descriptive statistics were calculated as means \pm SD. The alpha level was set at $P < 0.05$.

4.3 Results

4.3.1 Indirect markers of muscle damage

Perceived muscle soreness ($F_{(2,14)} = 80.2$, $P \leq 0.0005$) and CK activity ($F_{(2,14)} = 7.3$, $P = 0.007$) increased over Time, being significantly higher at both 24 and 48 h after the squatting exercise. Peak knee extensor torque decreased over Time ($F_{GG(1.2,8.4)} = 8.7$, $P = 0.015$), with values at 24 and 48 h being lower than baseline (Figure 4.2).

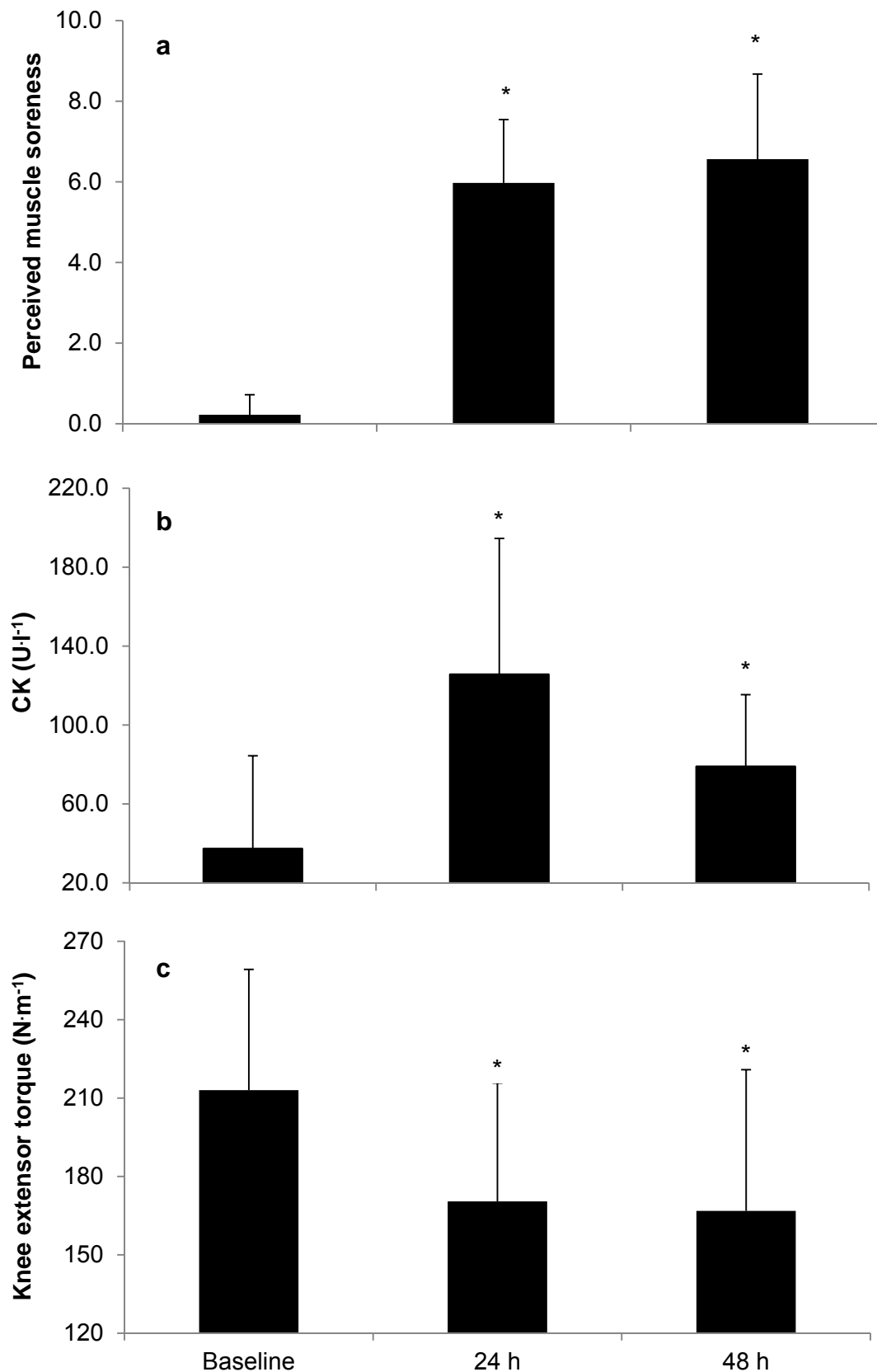


Figure 4.2. Changes in **a** perceived muscle soreness, **b** CK and **c** knee extensor torque after squatting exercise. Values are \pm means SD. * significantly different from baseline ($P < 0.05$).

4.3.2 The effects of muscle damage on resting metabolism

Resting $\dot{V}O_2$ ($F_{GG(1.1,7.9)} = 25.2$, $P = 0.001$), RMR ($F_{(2,14)} = 24.4$, $P = 0.0005$) and resting HR ($F_{(2,14)} = 7.2$, $P = 0.007$) increased over Time, being significantly elevated above baseline at both 24 and 48 h (see Table 4.2). However, no change in resting RQ ($F_{GG(1.2,8.4)} = 0.12$, $P = 0.889$) was observed after muscle damage.

Table 4.2. Mean (\pm SD) changes in resting metabolic rate after muscle damage.

	Baseline	24 h	48 h
Resting $\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	3.4 \pm 0.2	3.9 \pm 0.2*	3.9 \pm 0.3*
RMR (kcal·d ⁻¹)	1852.3 \pm 327.8	2070.7 \pm 286.1*	2097.1 \pm 322.3*
Resting HR (b·min ⁻¹)	54 \pm 5	58 \pm 5*	57 \pm 4*
Resting RQ	0.85 \pm 0.04	0.85 \pm 0.08	0.84 \pm 0.04

* significantly different from baseline ($P < 0.05$). *Abbreviations:* $\dot{V}O_2$ = oxygen uptake, RMR = resting metabolic rate, HR = heart rate, RQ = respiratory quotient.

4.3.3 Sub-maximal exercise responses to muscle-damaging exercise

All physiological, metabolic and perceptual responses during sub-maximal running were increased over Time ($\dot{V}O_2$ ($F_{(2,14)} = 5.3$, $P = 0.019$); \dot{V}_E ($F_{(2,14)} = 9.4$, $P = 0.003$); f_R ($F_{(2,14)} = 9.8$, $P = 0.002$); [La] ($F_{(2,14)} = 4.3$, $P = 0.034$); HR ($F_{(2,14)} = 6.8$, $P = 0.009$); RPE ($F_{(2,14)} = 5.7$, $P = 0.016$), and in particular were raised from baseline at 24 and 48 h (see Table 4.3).

Table 4.3. Mean (\pm SD) physiological, metabolic and perceptual responses during sub-maximal running after muscle-damaging exercise.

	Baseline	24 h	48 h
$\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	46.2 \pm 3.2	48.5 \pm 3.9*	48.1 \pm 3.6*
\dot{V}_E (l·min ⁻¹)	103.8 \pm 25.3	117.9 \pm 31.7*	115.9 \pm 29.5*
f_R (breaths·min ⁻¹)	40.5 \pm 7.9	46.8 \pm 9.8*	46.0 \pm 8.3*
[La] (mmol·l ⁻¹)	4.8 \pm 1.7	5.5 \pm 2.1*	5.1 \pm 1.9*
HR (b·min ⁻¹)	170 \pm 9	175 \pm 6*	174 \pm 7*
RPE	14.8 \pm 1.4	16.0 \pm 1.5*	16.1 \pm 1.7*

* significantly different from baseline ($P < 0.05$).

4.3.4 The effects of muscle damage on post-exercise oxygen consumption

Total ($F_{(2,14)} = 8.6$, $P = 0.004$) and mean ($F_{(2,14)} = 7.2$, $P = 0.007$) recovery $\dot{V}O_2$, EPOC ($F_{(2,14)} = 8.6$, $P = 0.004$), recovery HR ($F_{(2,14)} = 9.0$, $P = 0.003$) and recovery \dot{V}_E ($F_{(2,14)} = 6.9$, $P = 0.008$) all increased across the repeated measurements, and were significantly greater at 24 and 48 h post muscle-damaging exercise (see Table 4.4). Mean recovery RQ, however, remained unaltered ($F_{GG(1.2,8.4)} = 3.8$, $P = 0.082$). To highlight the changes in resting, exercise and recovery $\dot{V}O_2$ before and 48 h after muscle-damaging exercise data are shown from a randomly selected participant in Figure 4.3.

Table 4.4. Mean (\pm SD) changes in post-exercise recovery after muscle-damaging exercise.

	Baseline	24 h	48 h
Total $\dot{V}O_2$ (l^{-1})	14.0 ± 2.9	$14.8 \pm 3.0^*$	$14.6 \pm 3.0^*$
Mean $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	6.0 ± 0.5	$6.3 \pm 0.7^*$	$6.2 \pm 0.7^*$
EPOC (l^{-1})#	5.9 ± 1.8	$6.8 \pm 2.1^*$	$6.6 \pm 2.1^*$
Recovery HR ($b \cdot min^{-1}$)	77 ± 8	$83 \pm 8^*$	$81 \pm 7^*$
Recovery RQ	0.92 ± 0.04	0.87 ± 0.07	0.89 ± 0.06
Recovery \dot{V}_E ($l \cdot min^{-1}$)	14.9 ± 3.0	$16.5 \pm 4.1^*$	$16.0 \pm 3.3^*$

* significantly different from baseline ($P < 0.05$). # EPOC calculated as total recovery $\dot{V}O_2$ - total baseline RMR $\dot{V}O_2$. Abbreviations: EPOC = excess post-exercise oxygen consumption.

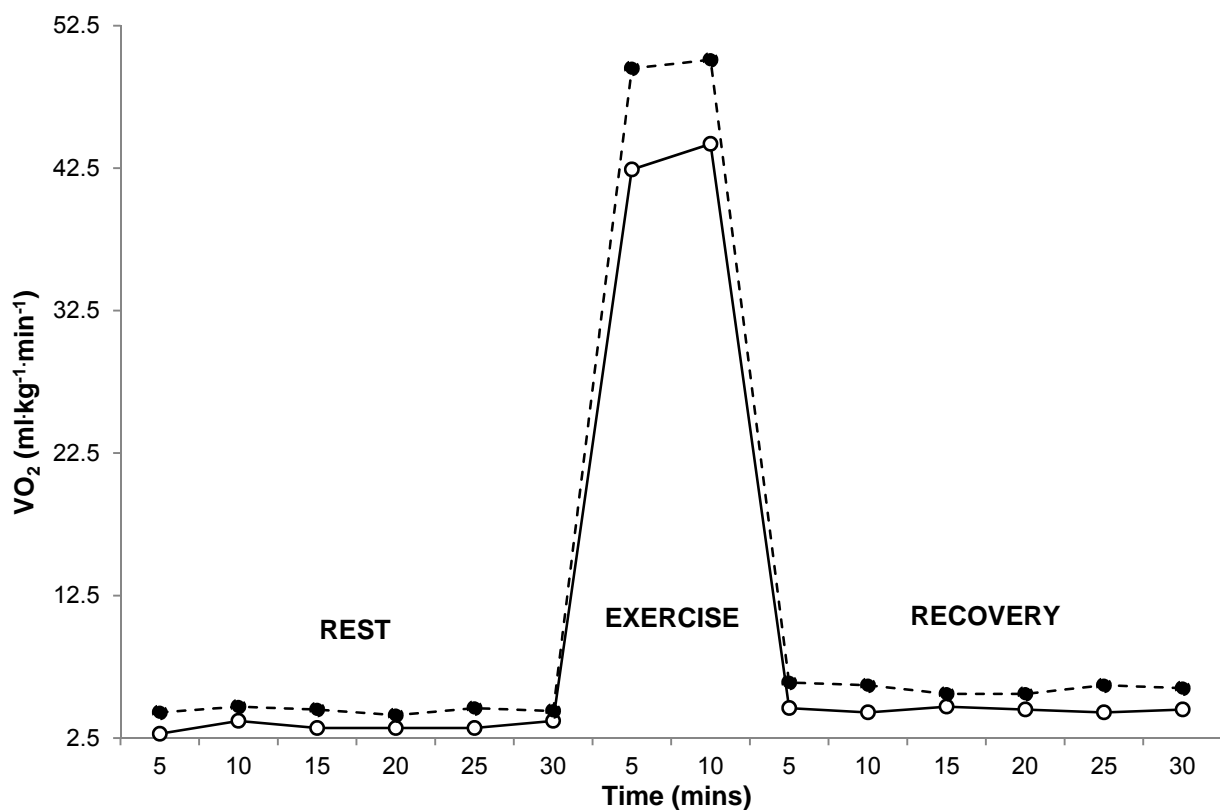


Figure 4.3. A time plot of $\dot{V}O_2$ during rest, exercise and recovery before (○) and 48 h after (●) exercise-induced muscle damage (data from a randomly selected participant).

4.4 Discussion

The efficacy of this study's attempt to create muscle damage via an unaccustomed bout of squatting exercise was confirmed by the notable changes in three indirect markers 24 and 48 h afterwards. As anticipated, the acute bout of resistance exercise was effective in elevating resting metabolism, with resting $\dot{V}O_2$, resting HR and RMR elevated above baseline values for the 48 h measurement period after EIMD. These findings concur with previous research reporting these increases for up to 15 – 72 h after similar exercise (Melby et al., 1993; Gillette et al., 1994; Dolezal et al., 2000; Schuenke et al., 2002; Jamurtas et al., 2004; Hackney et al., 2008; Paschalis et al., 2010; 2011). Observations do contradict the findings of Thomas et al. (1994) and Kolkhorst et al. (1994) who reported an unchanged RMR 24 h after muscle-damaging exercise, although in both cases a failure to assess the extent of muscle damage undermined their findings.

The increase in RMR observed after an acute bout of eccentric-biased exercise is attributed to the appearance of muscle damage and the stimulus for repair (Burleson et al., 1998; Dolezal et al., 2000; Paschalis et al., 2010). It is well established that muscle damage is accompanied by an inflammatory response that causes the influx of neutrophils and macrophages at the site of injury (Smith, 1991; MacIntyre et al., 1995; Sorichter et al., 1999), the purpose of which is to promote the degradation and synthesis of damaged muscle fibres (MacIntyre et al., 1995). The energy cost of protein synthesis can account for as much as 20% of total resting metabolism (Welle & Nair, 1990). Furthermore, in adults with severe burn trauma, protein synthesis correlates positively with oxygen uptake and RMR (Cunningham et al., 1989). Therefore, it is plausible that the stimulus for repair after unaccustomed resistance exercise led to the increase in RMR observed in the current study.

Alternatively, the prolonged increase in RMR after muscle damage could have been caused by an elevation in sympathetic nervous system activity. Moreau et al. (1995) reported increased concentrations of urinary norepinephrine and epinephrine 24 – 48 h after muscle-damaging exercise. The authors posited that the stress associated with movement of sore muscles led to the release of catecholamines from the adrenal medulla, which acts to increase the rate and strength of cardiac contraction, and might explain the increase in HR observed before, during and after endurance exercise. Furthermore, activation of the sympathetic nervous system causes vasodilation of the blood vessels that supply skeletal muscle, which could have accounted for the greater $\dot{V}O_2$ before, during and post-exercise (Sherwood, 2001; Schuenke et al., 2002).

Surprisingly, there was no change in resting substrate utilisation after muscle damage. In using the respiratory quotient (RQ) to provide an indirect measure of substrate use, a decrease infers that fat utilisation is increased in the days after EIMD (Schuenke et al., 2002; Jamurtas et al., 2004; Paschalis et al., 2010; 2011). Muscle damage also increases insulin resistance (Tee et al., 2007), reduces glucose disposal rates (Kirwan et al., 1992) and decreases glucose transporter (GLUT-4) protein concentration (Asp et al., 1995). Therefore, changes to the glucose transport system might explain the reliance on fat during recovery from EIMD (Paschalis et al., 2010). Alternatively, damage to the muscle membrane could breakdown fatty acid phospholipids, increasing beta oxidation due to the greater availability of free fatty acid inside the muscle cell (Paschalis et al., 2010). However, that other studies reported a decrease in resting RQ and the current study did not is possibly owing to the type of muscle-damage protocols employed. The use of whole-body resistance exercise (Schuenke et al., 2002; Jamurtas et al., 2004) and isokinetic eccentric

exercise (Paschalis et al., 2010) might have imposed greater changes in resting RQ than squatting exercise.

This is the first study to examine the impact of muscle damaging exercise on EPOC after endurance exercise, where total $\dot{V}O_2$ was 4 – 6% higher during recovery from sub-maximal running whilst participants were experiencing symptoms of muscle damage. In the only comparable study, Bahr et al. (1991) investigated recovery from 3 – 4 days of strenuous continuous training, after which participants underwent 60 minutes of supine rest to determine RMR, 30 minutes of cycling at 50% $\dot{V}O_{2\max}$ and a further 60 minutes of supine rest after exercise to determine EPOC. In comparison to a control condition, total oxygen consumption during recovery after cycling exercise was 24% higher after strenuous training. Direct comparisons between the current study and the findings of Bahr et al. (1991) are difficult due to differences in the type of endurance exercise (running versus cycling), the timing of EPOC (0.5 hour versus 1 hour) and the stress conditions (single resistance training session versus 3 – 4 days of intensive training). However, our findings reaffirm those of Bahr and colleagues that engaging in further exercise after a period of intensified training causes an increase in EPOC.

It was shown in Chapter 3 that EIMD increases the oxygen cost required during subsequent sub-maximal endurance exercise. These changes have been attributed to alterations in running stride pattern (Braun & Dutto, 2003), an inability to store and use elastic energy (Chen et al., 2007b; 2009) and an increase in motor unit recruitment in order to produce the same pre-damage force (Kyrolainen et al., 2000).

This study reaffirms the findings from Chapter 3, with the observed 4 – 5% increase in oxygen uptake during sub-maximal running (after EIMD) indicating that

participants were exercising, on average, at 89 – 90 % $\dot{V}O_{2\max}$ compared to 85% at baseline. Furthermore, it is likely that this greater energetic cost during sub-maximal running after EIMD is responsible for the observed increase in EPOC (Gore & Withers, 1990a; 1990b; Bahr & Sejersted, 1991; Bahr, 1992; Smith & McNaughton, 1993).

Blood lactate responses at the end of sub-maximal running were approximately 6 – 14% higher than baseline in the days after muscle-damaging exercise. This increase is in agreement with other studies (Gleeson et al., 1995; Braun & Dutto, 2003; Chen et al., 2007b; 2009; Schneider et al., 2007) and reflects an increased recruitment of non-damaged type II fibres in order to maintain pre-damage capacities. It is also possible that the increase in [La] response contributed slightly to the elevated EPOC observed after sub-maximal running. Research has shown that only a small fraction of recovery oxygen is required for the reconversion of lactate to glycogen (Gaesser & Brooks, 1984; Bangsbo et al., 1991; Bahr, 1992). Hill & Lupton (1923) first hypothesised that 80% of the lactate produced during exercise was reconverted to glycogen, with the remaining 20% being oxidised to provide the energy required for glycogen resynthesis. However, evidence has shown that less than 20% of the post-exercise oxygen consumption is responsible for the reformation of glycogen from lactate (Gaesser & Brookes, 1984; Bangsbo et al., 1991).

Other mechanisms that contribute to the EPOC include the replenishment of myoglobin and haemoglobin and the resynthesis of adenosine tri-phosphate (ATP) and creatine phosphate (CP) (Gaesser & Brooks, 1984; Bahr, 1992; Bangsbo & Hellsten, 1998; Borsheim et al., 1998a). Indeed, Davies et al. (2011b) recently reported an increase in resting inorganic phosphate concentration ([Pi]) after EIMD,

which they attributed to an increase in the ATP turnover required for the repair and remodelling of damaged tissue. A sustained increase in ventilation has also been used to explain EPOC (Bahr, 1992; Bangsbo & Hellsten, 1998; Borsheim et al., 1998a). In this study, ventilatory responses during exercise and recovery were higher than baseline at 24 and 48 h after muscle-damaging exercise. Given that group III and IV afferents located in and around skeletal muscle are responsible for controlling ventilation (Haouzi et al., 2004), it is hypothesised that damage to the muscle stimulates a discharge from the nerve afferents causing ventilation to increase (Hotta et al., 2006; Davies et al., 2009; Twist & Eston, 2009). Any residual muscle pain experienced after sub-maximal running might have also provided a stimulus to increase ventilation through activating nociceptive muscle afferents (Duranti et al., 1991). More recently, research has attributed elevations in oxygen demand during exercise to an increase in ventilation (Burt & Twist, 2011). Therefore, it is possible that oxygen demand during recovery was increased to facilitate the increased ventilation because of damaged muscle tissue.

Heart rates were also increased during recovery from sub-maximal running in the days after EIMD. The return of heart rate to resting values after exercise has a similar time course to EPOC (Bahr, 1992). It is plausible that the stress associated with the movement of sore muscles caused the secretion of catecholamines and subsequent elevation in heart rate during the recovery period (Moreau et al., 1995).

Catecholamines also regulate the triglyceride / fatty acid (TG / FA) cycle and fat oxidation through stimulating β -adrenoceptors (Borsheim et al., 1998a; 1998b). During the TG / FA cycle, FA released from lipolysis is re-esterified into TG rather than being oxidised, and it is the increase in ATP required for this process that is thought to contribute to EPOC (Borsheim & Bahr, 2003). Since fat oxidation requires

relatively more oxygen than carbohydrate oxidation, any shift from carbohydrate to fat utilisation during recovery would also contribute to EPOC (Borsheim et al., 1998a; 1998b). Although not statistically significant, the recovery RQ was lower than baseline in the current study, suggesting a greater proportion of fat was utilised after sub-maximal running when muscle damage was present.

The possibility that an elevated RMR after the muscle-damaging (squatting) exercise had a residual effect on EPOC after the sub-maximal endurance exercise can also not be ruled out. That is, the elevated rate of protein breakdown and resynthesis after muscle-damaging exercise could have contributed to the increase in oxygen uptake both during and after sub-maximal running exercise.

Whilst it might be difficult to extrapolate our findings to those of others who have used a larger sample size, our findings are in agreement with previous studies that used larger sized samples. However, future research should investigate if RMR and EPOC are elevated after muscle-damaging exercise in a larger sample size and a control group should also be used to further reduce the risk of a type I error. It is accepted that there were limitations with the assessment of dietary intake during data collection. The variability of our diet from a day-to-day basis questions whether the 24 h recorded before data collection was a true representation of diet. Furthermore, instructing participants to maintain a similar energy intake throughout the study may have been laborious. To address these limitations, participants were asked to complete the food diary over a typical day and were provided with detailed instructions on how to complete the diary and the importance of maintaining a similar diet throughout the investigation.

The capacity of resistance exercise to induce further improvements in running economy is alluring to endurance runners. However, as this and the previous study have shown, performing unaccustomed resistance exercise causes the acute symptoms of muscle damage. The results of this study revealed that in the days after muscle-damaging exercise RMR is increased by 11.8 – 13.2%. To compensate for this increase and in order to maintain a positive energy balance between training sessions individuals may need to increase their calorie intake. However, for individuals trying to facilitate a negative energy balance, increasing resting energy expenditure through unaccustomed resistance exercise could provide a short-term health-promoting effect. Endurance athletes often engage in repeated bouts of endurance exercise on the same day or in the days after. The increased oxygen demand during endurance running after muscle damage led to an increase in EPOC. On this basis, individuals should periodise their recovery after unaccustomed resistance exercise to preserve their exercise economy during subsequent training.

In conclusion, findings from previous research and Chapter 3 infer that EIMD increases oxidative metabolism at rest and during endurance exercise. Nevertheless, it was not known whether oxygen uptake during recovery from endurance exercise is also augmented when experiencing EIMD. Thus, the aim of this study was to investigate the effects of EIMD on $\dot{V}O_2$ before, during and after sub-maximal running. In order to address this aim, participants completed baseline measurements comprising RMR, sub-maximal running and 30 minutes recovery to ascertain EPOC. Measurements were then repeated 24 and 48 h after muscle-damaging exercise. This is the first study to demonstrate that in the presence of EIMD, oxygen uptake is increased at rest, during and after endurance exercise. The

elevated RMR in the days after EIMD is in agreement with research and reflects the breakdown and repair of damaged muscle that is energetically expensive. The higher oxygen cost during sub-maximal running highlights that for a given exercise intensity participants are working harder than without EIMD. Moreover, this is the first study to show an increase in EPOC after sub-maximal running whilst experiencing EIMD. This is likely to be a direct consequence of the greater energetic demand of running observed when muscle-damaged. Individuals engaging in unaccustomed resistance exercise that results in muscle damage should be mindful of the increases in resting energy expenditure and increased metabolic demand to exercise in the days that follow. The next aim of this thesis will be to investigate whether the same changes in endurance exercise are evident after a second bout of EIMD, since the signs and symptoms of muscle damage appear to be attenuated.

CHAPTER 5

THE EFFECTS OF REPEATED BOUTS OF MUSCLE DAMAGING EXERCISE ON PHYSIOLOGICAL, METABOLIC, PERCEPTUAL AND KINEMATIC RESPONSES DURING SUB-MAXIMAL RUNNING

The contents of this chapter have previously been presented / published in the following:

Burt, D.G., Lamb, K., Nicholas, C., & Twist, C. (2013). Effects of repeated bouts of squatting exercise on sub-maximal endurance running performance. *European Journal of Applied Physiology*, 113(2), 285-293.

Burt, D.G., Twist, C., Lamb, K., & Nicholas, C. (2011). The effects of repeated bouts of muscle-damaging exercise on physiological and perceptual responses during sub-maximal running. The Annual Student Conference of the British Association of Sport and Exercise Science, Chester, UK.

Burt, D.G., Twist, C., Lamb, K., & Nicholas, C. (2011). The effects of repeated bouts of muscle-damaging exercise on physiological, perceptual and kinematic responses during sub-maximal running. The Annual Conference of the British Association of Sport and Exercise Science, Essex, UK.

5.1 Introduction

The provision of resistance training alongside endurance training (also referred to as concurrent training) is known to improve running economy (Guglielmo et al., 2009; Storen et al., 2008; Millet et al., 2002; Johnston et al., 1997). Furthermore, as shown in Chapter 4, resistance exercise also induces health-promoting effects, such as elevating resting energy expenditure. However, as demonstrated in Chapters 3 and 4, a consequence of resistance exercise, when novel and unaccustomed, is the immediate and prolonged appearance of symptoms associated with EIMD. These symptoms include muscle soreness, swelling and reduced muscle strength (Byrne et al., 2004), and at their peak, typically 24 - 48 h after muscle-damaging exercise, have been found to impact negatively on sub-maximal running performance. Indeed, findings from Chapters 3 and 4 infer that oxygen demand for a given running intensity (i.e. running economy) is increased after EIMD. This elevation is attributed to altered gait kinematics (Braun & Dutto, 2003), an impaired ability to absorb and utilize elastic energy (Chen et al., 2007b; 2009) and increased motor unit recruitment (Kyrolainen et al., 2000).

Likewise, increases in [La] during sub-maximal running after EIMD were also observed in Chapter 4, and are attributed to an increase in the recruitment of non-damaged type II fibres in an attempt to maintain pre-damage capacities (Braun & Dutto, 2003). Since type II fibres are more glycolytic, an increase in their activation after EIMD is likely to result in an increased [La] during endurance exercise (Gleeson et al., 1998; Scott et al., 2003). Whilst Scott et al. (2003) have reported unchanged [La] during sub-maximal running (deemed to be below the LT), work by Chen et al. (2009) confirmed that the increased [La] after EIMD is more pronounced at exercise intensities above the LT. It was suggested that because type I fibres are

predominantly recruited at intensities below the LT, and are less susceptible to EIMD (Friden et al., 1983), these fibres are capable of maintaining a normal recruitment pattern and metabolic function (Scott et al., 2003).

Increases in effort perception have also been observed during sub-maximal endurance exercise after muscle-damaging exercise (as shown in Chapters 3 and 4), and are associated with the increased muscle pain accompanying EIMD (Scott et al., 2003). Furthermore, as reported in Chapter 3, increases in RPE coincided with increases in \dot{V}_E , which may be manifested by the activation of nerve afferents located in and around the blood vessels of the exercising muscles that are involved in controlling ventilation (Hotta et al., 2006; Twist & Eston, 2009; Davies et al., 2011).

However, after an initial bout of muscle-damaging exercise, adaptation occurs to the muscle, whereby if the same bout of eccentric exercise was repeated it would result in the symptoms of EIMD being attenuated (McHugh et al., 1999a; McHugh, 2003; Howatson & van Someren, 2008). This protective adaptation is referred to as the 'repeated bout effect' (RBE) and is characterized by a reduced deficit in muscle function, a lowered perception of muscle soreness, and a reduction in the leakage of myofibre proteins into the circulation (Byrnes et al., 1985; Newman et al., 1987; Nosaka & Clarkson, 1995; Hortobagyi et al., 1998; Howatson et al., 2007; Smith et al., 2007). The exact mechanism responsible for the RBE has not been established, however several studies suggest that the protective adaptation is attributed to either neural or peripheral mechanisms (McHugh et al., 1999a; McHugh, 2003). The neural theory contends that EIMD is attenuated through a more efficient recruitment of motor units allowing for a greater distribution of contractile stress across a larger number of fibres (Golden & Dudley, 1992; Nosaka & Clarkson, 1995), an increased

motor unit synchronization or an increase in slow-twitch muscle fibre recruitment (Warren et al., 2000; Chen, 2003; Howatson et al., 2007; Starbuck & Eston, 2012). Alternatively, the periperal theory proposes that the RBE resides within the muscle through an increase in connective tissue (Lapier et al., 1995), the remodelling of intermediate filaments (Lehti et al., 2007), strengthening of the muscle cell membrane (Clarkson & Tremblay, 1988), removal of weak muscle fibres (Newham et al., 1987), longitudinal addition of sarcomeres (Brockett et al., 2002), changes to E-C coupling (Clarkson & Tremblay, 1988) or an attenuated inflammatory response (Pizza et al., 1996).

Whilst findings from Chapter 3 and 4 show that an initial bout of muscle-damaging exercise alters the physiological, metabolic, perceptual and kinematic responses during sub-maximal endurance exercise, the impact after a repeated bout of damaging exercise is yet to be elucidated. Therefore, this study aimed to 1) confirm that symptoms (muscle soreness, increased CK activity and decreased muscle function) of EIMD that follow an initial bout of squatting exercise are attenuated when the same bout of muscle-damaging exercise is performed two weeks later; 2) to reaffirm that $\dot{V}O_2$, \dot{V}_E , [La], RPE, SL and SF during sub-maximal running are altered as a result of EIMD; and 3) to investigate whether these responses are attenuated during sub-maximal running after a repeated exposure to the muscle-damaging exercise. Considering that individuals will engage in numerous resistance training sessions, rather than a single bout, to induce adaptations in endurance performance it appears imperative to examine whether responses during sub-maximal endurance exercise are still altered after a repeated bout of resistance exercise.

5.2 Methods

5.2.1 Participants

Nine healthy male participants (age 25.8 ± 4.1 years, stature 1.80 ± 0.09 m, body mass 79.6 ± 9.9 kg), who all engaged in regular physical activity (2-3 endurance exercise sessions per week), but had not undertaken any form of lower limb resistance exercise in the previous six months, volunteered to participate in the study. Their physiological characteristics are shown in Table 1. Before the study, each participant completed a written informed consent form and medical health questionnaire and received verbal explanation of the risks associated with the experimental procedures. Institutional ethical approval was obtained from the Faculty of Applied Sciences Research Ethics Committee.

5.2.2 Study Design

The participants took part in a repeated measures design involving seven laboratory visits over a period of 5 weeks (see Figure 5.1). An initial exhaustive incremental running trial to establish individual LTP and $\dot{V}O_{2\text{ peak}}$ was performed, followed by habituation to the procedures used to measure perceived muscle soreness, isokinetic strength, and vertical jump performance (Visit 1). This was followed 24 – 72 h later by baseline measurements of perceived muscle soreness, isokinetic strength, CK, vertical jump performance and a bout of sub-maximal running at a speed corresponding to the individual's LTP. During the same visit, participants experience a bout of lower limb resistance exercise designed to elicit muscle damage (Visit 2). Measurements were then repeated 24 h and 48 h after the EIMD (Visits 3 and 4). Two weeks later, participants returned to the laboratory to repeat the baseline measurements and the same muscle-damaging exercise (Visit 5). Two

further testing sessions then occurred 24 and 48 h later (Visits 6 and 7). All participants were asked to refrain from any strenuous exercise 24 h prior to each visit, maintain their normal diet, and avoid using any analgesic agents.

Table 5.1. Participants' physiological characteristics

	Mean \pm SD
$\dot{V}O_{2\text{ peak}}$ (ml·kg ⁻¹ ·min ⁻¹)	54.2 \pm 3.2
HR _{peak} (b·min ⁻¹)	190 \pm 9
LTP (mmol·l ⁻¹)	4.7 \pm 0.8
LTP _{SPEED} (km·h ⁻¹)	12.3 \pm 0.8
<i>Abbreviations:</i> $\dot{V}O_{2\text{ peak}}$ = peak oxygen uptake; HR _{peak} = peak heart rate at peak oxygen uptake; LTP = lactate turnpoint; LTP _{SPEED} = speed corresponding to lactate turnpoint.	

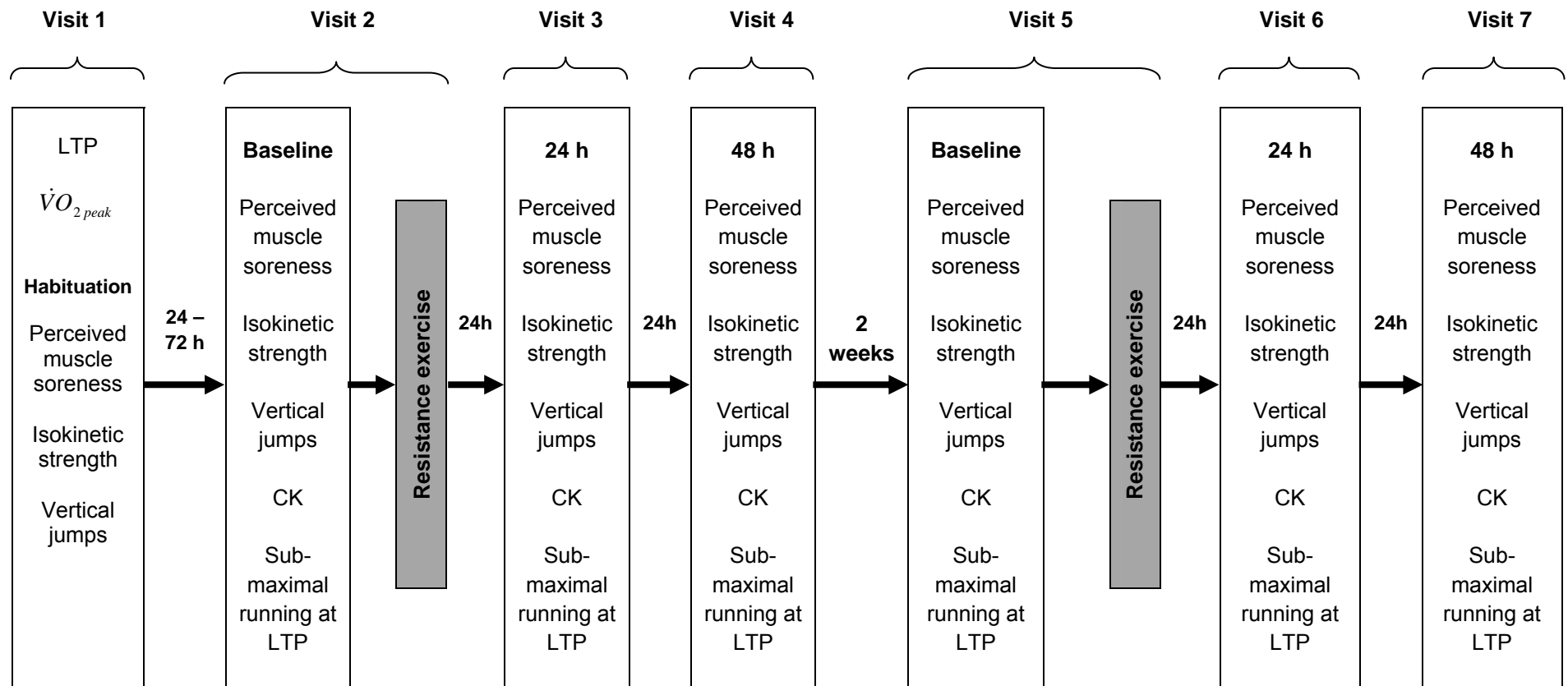


Figure 5.1. Schematic of the study design

5.2.3 Assessment of lactate turnpoint and peak oxygen uptake

Participants performed the same incremental protocol to exhaustion as previously detailed in Chapter 4.

5.2.4 Perceived muscle soreness

Each participant was asked to provide his perceived level of muscle soreness of the knee extensors using the same instructions outlined in Chapter 3.

5.2.5 Peak knee extensor torque

Knee extensor torque was measured on the dominant limb using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). The same testing guidelines are detailed in Chapter 3.

5.2.6 Vertical jump performance

Participants were asked to perform separate trials of squat (SJ), countermovement (CMJ) and drop jumps (DJ), which were recorded using an infra-red timing system (Optojump, Microgate S.r.l, Bolzano, Italy). After warm-up trials, each jump was performed three times, in the same order and with 60 s rest between each jump. Maximal jump height was then recorded and used for analysis. The SJ was performed with the participant starting in a crouched position, with knees flexed at 90° before jumping for maximal height. The CMJ started with the participant in an upright position after which they were required to rapidly flex the knees to 90° before jumping for maximal height. The DJ was performed from a height of 50 cm (Horita et al., 1999) and required participants to drop to the ground and after minimal contact jump for maximal height. Vertical jump height was calculated for each jump from the

method previously used by Byrne and Eston (2002a) and Twist and Eston (2007). Measuring flight time (t_{flight}) enabled vertical take-off velocity (v) to be calculated as: $v = 0.5 (t_{\text{flight}} \times g)$, where g is the acceleration due to gravity ($9.81 \text{ m}\cdot\text{s}^{-2}$). Vertical jump height was then calculated from the equation: $\text{height} = v^2 / 2g$. Reliability data for the SJ, CMJ and DJ demonstrated a CV of 2.3%, 2.6% and 3.4% respectively.

5.2.7 Determination of creatine kinase

Plasma blood CK activity was assessed using the same method described in Chapter 3.

5.2.8 Sub-maximal running protocol

Sub-maximal running was performed on a motorized treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany) at a speed corresponding to previously determined individual LTPs for 10 minutes. Expired air, HR, RPE, and [La] were recorded as detailed in Chapter 3.

5.2.9 Running stride pattern

A high speed video camera (Casio Exilim, Pro Ex-F1) recorded sagittal plane images at 100 Hz during the final 10 s of each 10-min sub-maximal running bout. Stride length and SF were then determined by the same methods previously described in Chapter 3.

5.2.10 Muscle-damaging exercise

To induce symptoms of muscle damage, participants performed the same bout of Smith-machine squats described in detail in Chapter 3. An identical bout of muscle-damaging exercise was also performed two weeks later.

5.2.11 Statistical analysis

The differences in the indirect markers of muscle-damage and sub-maximal running performance between bouts were analysed using separate two-way (Time [3] and Bout [2]) repeated measures ANOVAs. The assumption of sphericity was analysed using Mauchly's test, with any violations adjusted by the use of the Greenhouse-Geisser correction. Where significant Time and Bout interaction effects were found, *post-hoc* Tukey tests modified for repeated measures (Stevens, 2002) were used to identify differences between conditions. Descriptive statistics were calculated as means \pm SD. The alpha level was set at $P \leq 0.05$.

5.3 Results

5.3.1 Indirect markers of muscle damage

Analysis revealed a significant Bout x Time ($F_{(2,16)} = 38.3$, $P \leq 0.0005$) interaction on muscle soreness, with post-hoc analysis indicating that muscle soreness was increased after both bouts of EIMD. However, significantly less soreness was reported in the days after Bout 2 when compared to Bout 1. The Bout x Time interaction ($F_{GG(1.1,8.6)} = 9.5$, $P = 0.013$) revealed that the notable decrements in peak torque 24 – 48 h after the first bout of EIMD did not occur after the second bout. The Bout x Time interactions on drop ($F_{GG(1.2,9.5)} = 11.5$, $P = 0.006$), countermovement ($F_{GG(1.2,9.9)} = 11.5$, $P = 0.005$) and squat ($F_{GG(1.1,8.9)} = 7.2$, $P = 0.023$) jump heights

were also significant, and characterized by vertical jump performance declining at 24 – 48 h after the initial bout of muscle damage, but being unchanged in the days after the repeated bout. Creatine kinase activity demonstrated a Bout effect ($F_{(1,8)} = 5.9$, $P = 0.042$), with values after Bout 1 being considerably greater than after Bout 2, however no significant Bout x Time interaction ($F_{(2,16)} = 2.4$, $P = 0.124$) was observed (Figures 5.2 – 5.3, Tables 5.2 – 5.3).

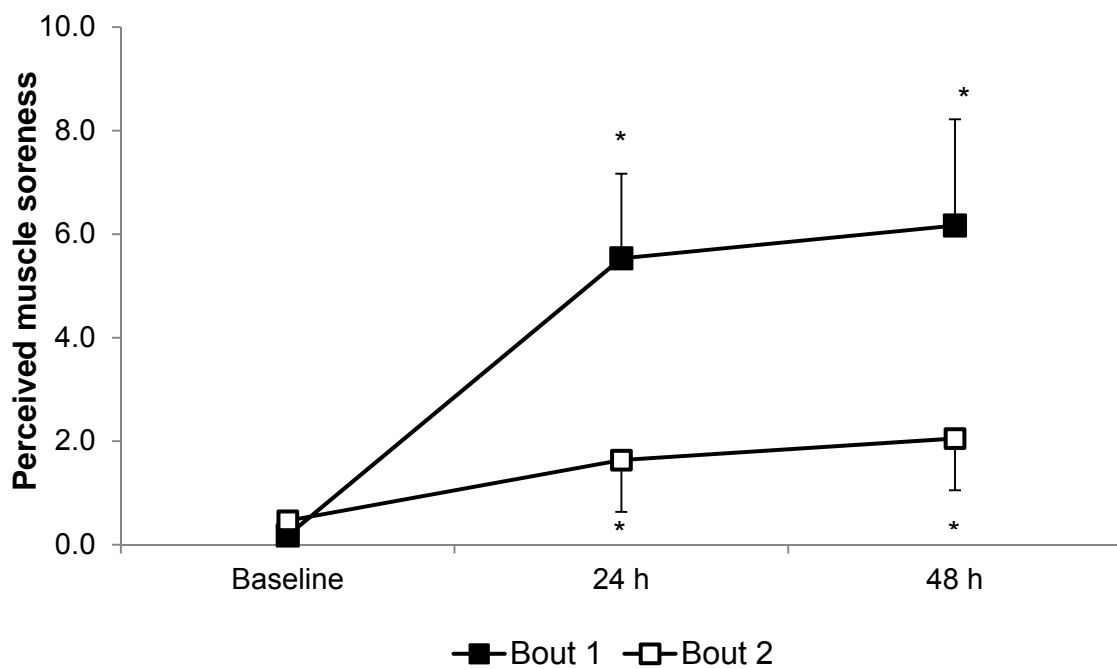


Figure 5.2. Changes in muscle soreness before (baseline) and 24 – 48 h after initial (Bout 1) and repeated (Bout 2) bouts of muscle damage. Values are shown as means \pm SD. * Significantly different from baseline ($P < 0.05$).

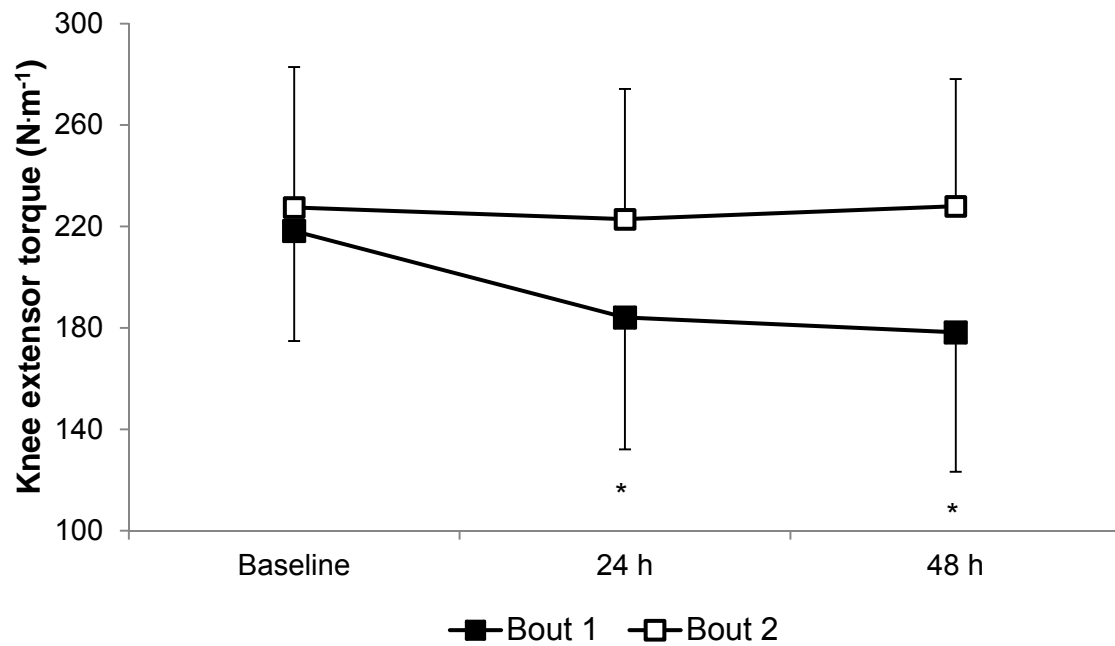


Figure 5.3. Changes in knee extensor torque before (baseline) and 24 – 48 h after initial (Bout 1) and repeated (Bout 2) bouts of muscle damage. Values are shown as means \pm SD. * significantly different from baseline ($P < 0.05$).

Table 5.2. Changes in vertical jump performance before (baseline) and 24 – 48 h after initial (Bout 1) and repeated (Bout 2) bouts of muscle damage. Values are shown as means \pm SD.

	Bout 1			Bout 2		
	Baseline	24 h	48 h	Baseline	24 h	48 h
DJ height (cm)	34.7 \pm 3.3	28.5 \pm 3.7*	29.0 \pm 5.2*	34.7 \pm 3.8	34.6 \pm 4	34.7 \pm 3.8
CMJ height (cm)	33.7 \pm 3.2	29.9 \pm 4.3*	28.7 \pm 5.9*	33.7 \pm 3.9	33.7 \pm 3.6	34.6 \pm 3.9
SJ height (cm)	31.2 \pm 3.3	26.8 \pm 4.6*	26.8 \pm 5.5*	30.5 \pm 3.5	29.6 \pm 3.7	30.6 \pm 3.9

* significantly different from baseline ($P < 0.05$). *Abbreviations:* DJ = drop jump, CMJ = counter movement jump, SJ = squat jump.

Table 5.3. Changes in CK activity before (baseline) and 24 – 48 h after initial (Bout 1) and repeated (Bout 2) bouts of muscle damage. Values are shown as means \pm SD.

	Bout 1#			Bout 2		
	Baseline	24 h	48 h	Baseline	24 h	48 h
CK (IU·l ⁻¹)	76.3 \pm 41.8	157.4 \pm 107.5	107 \pm 81.6	59.3 \pm 27.8	70.1 \pm 33.2	63.9 \pm 43.5

significant overall difference between Bout 1 and Bout 2 ($P < 0.05$).

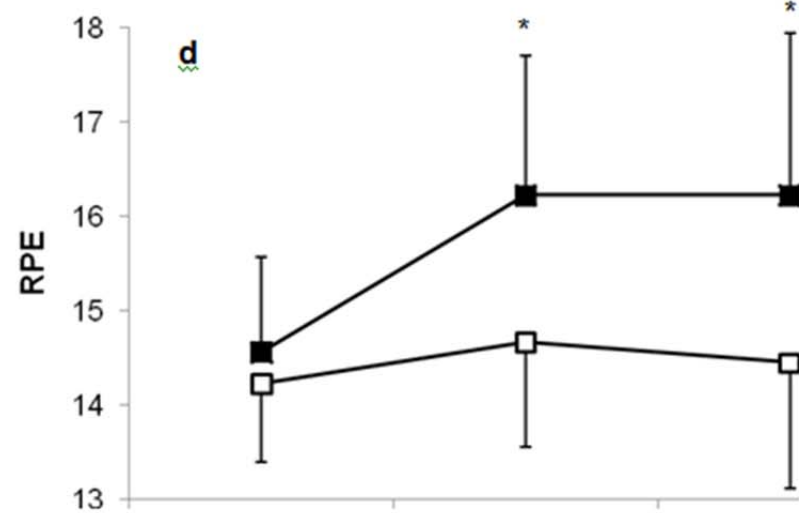
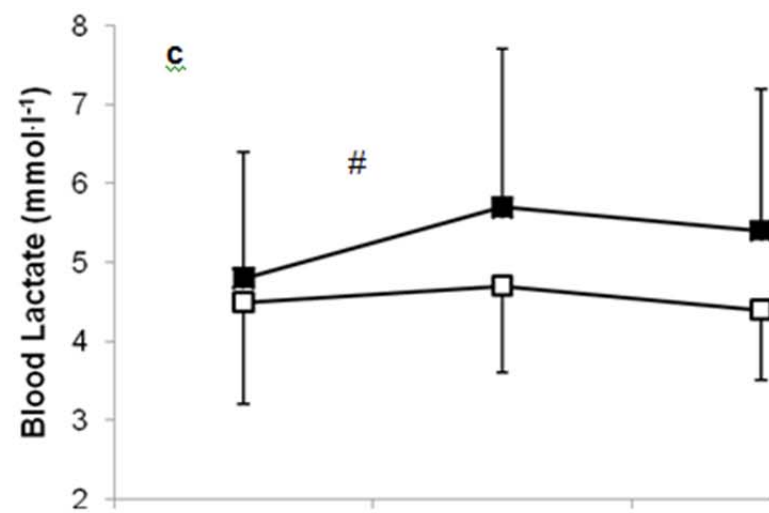
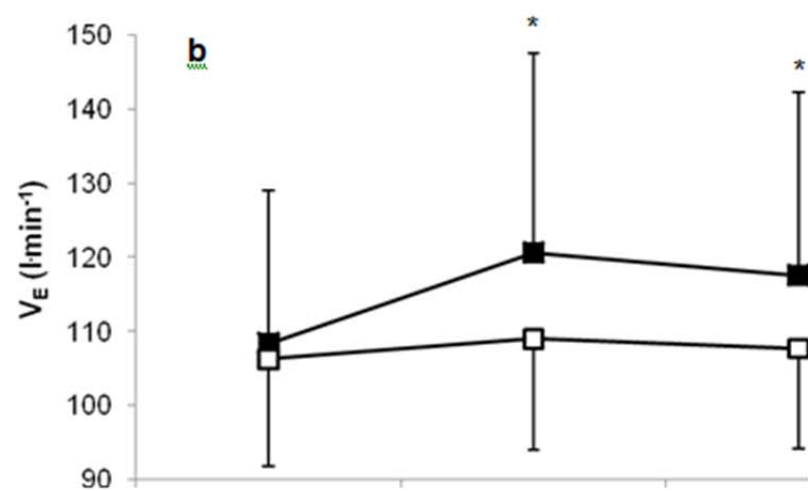
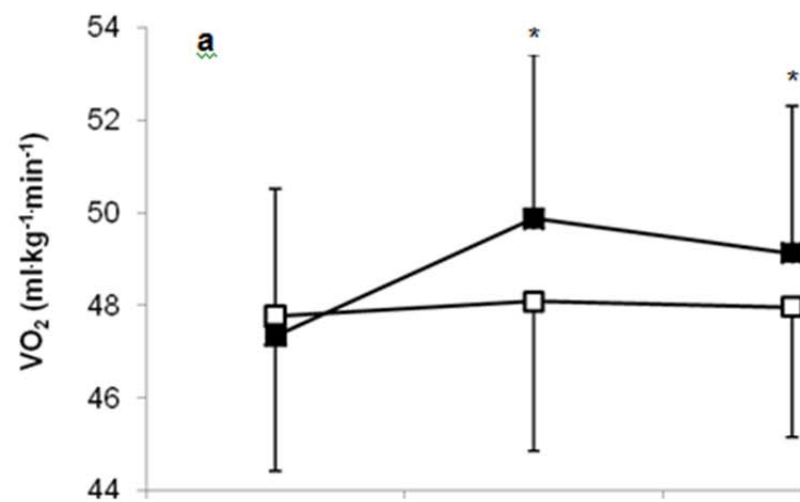
5.3.2 Sub-maximal running responses to repeated bouts of muscle damage

Figure 5.4 and Table 5.4 highlights significant Bout x Time interactions in most of the measures presented. That is, for $\dot{V}O_2$ ($F_{(2,16)} = 7.7$, $P = 0.005$) it is clear that the increases evident 24 – 48 h after Bout 1 no longer occurred in the days after Bout 2. The same pattern of variability was seen for \dot{V}_E ($F_{(2,16)} = 5.0$, $P = 0.021$), RPE ($F_{(2,16)} = 8.3$, $P = 0.003$), f_R ($F_{(2,16)} = 8.9$, $P = 0.003$), HR ($F_{GG(1.3,10)} = 5.4$, $P = 0.037$), SL ($F_{(2,16)} = 5.9$, $P = 0.012$) and SF ($F_{GG(1.2,9.5)} = 8.7$, $P = 0.013$), but not for [La] ($F_{(2,16)} = 2.7$, $P = 0.101$), $\dot{V}_E / \dot{V}O_2$ ($F_{(2,16)} = 1.7$, $P = 0.213$) or $\dot{V}_E / \dot{V}CO_2$ ($F_{(2,16)} = 2.1$, $P = 0.154$). A significant effect of Bout for [La] ($F_{(1,8)} = 9.0$, $P = 0.017$), $\dot{V}_E / \dot{V}O_2$ ($F_{(1,8)} = 5.4$, $P = 0.049$) and $\dot{V}_E / \dot{V}CO_2$ ($F_{(1,8)} = 6.6$, $P = 0.034$) was observed, reflecting higher overall values after Bout 1 compared to Bout 2.

Table 5.4. Changes in stride pattern before (baseline) and 24 – 48 h after initial (Bout 1) and repeated (Bout 2) bouts of muscle damage. Values are shown as means \pm SD.

	Bout 1			Bout 2		
	Baseline	24 h	48 h	Baseline	24 h	48 h
SL (m)	2.45 \pm 0.25	2.39 \pm 0.24*	2.39 \pm 0.23*	2.44 \pm 0.26	2.46 \pm 0.24	2.46 \pm 0.24
SF	14.0 \pm 0.8	14.4 \pm 0.6*	14.4 \pm 0.6*	14.0 \pm 0.8	14.0 \pm 0.8	14.0 \pm 0.8

* significantly different from baseline ($P < 0.05$)



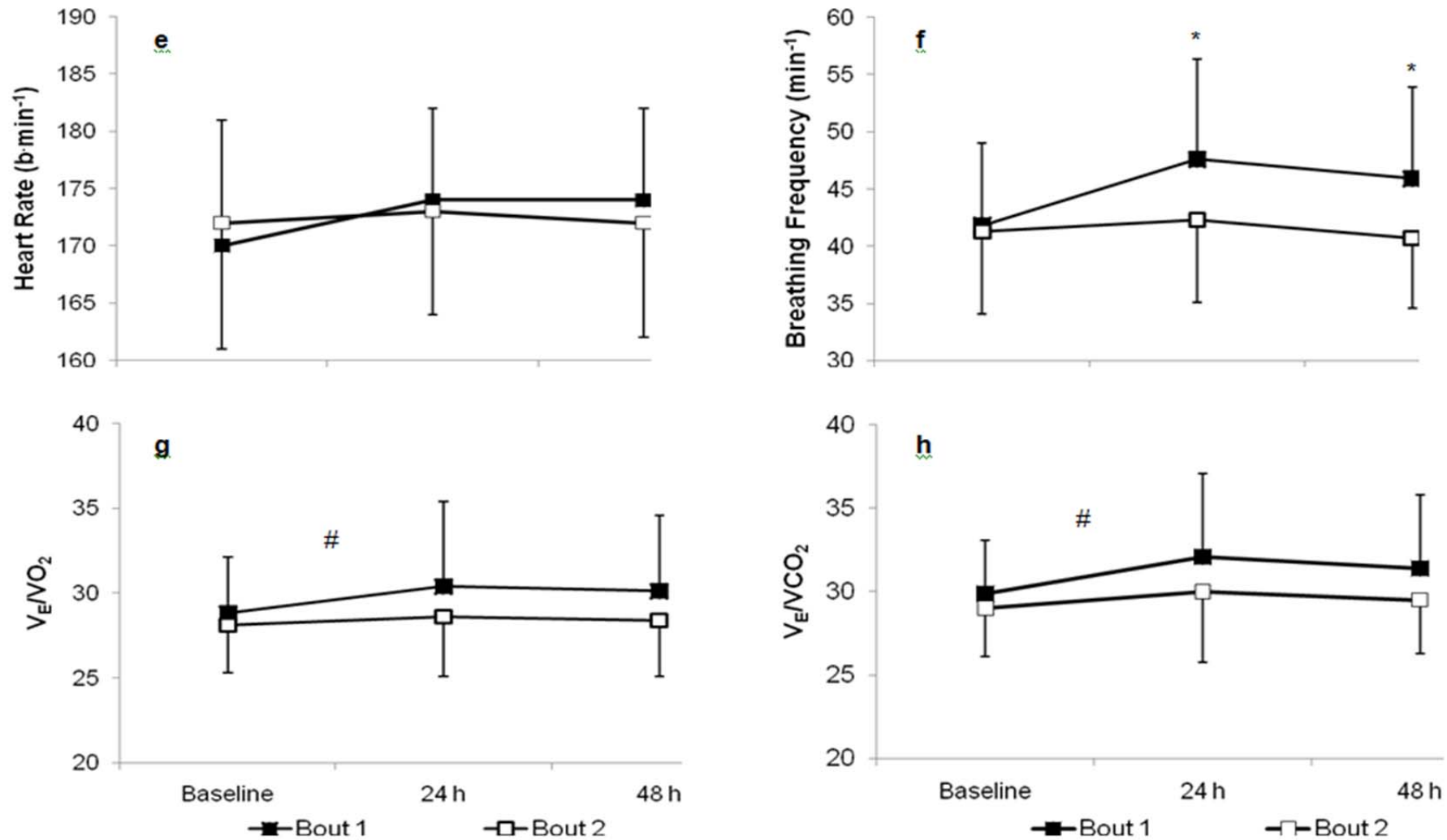


Figure 5.4. Changes in **a** oxygen uptake (\dot{V}_{O_2}), **b** ventilation (\dot{V}_E), **c** blood lactate, **d** RPE, **e** heart rate, **f** breathing frequency, **g** ventilatory equivalent for oxygen (\dot{V}_E/\dot{V}_{O_2}), and **h** ventilatory equivalent for carbon dioxide (\dot{V}_E/\dot{V}_{CO_2}) before (baseline) and 24 – 48 h after initial (Bout 1) and repeated (Bout 2) bouts of muscle damage. Values are shown as means \pm SD. * significantly different from baseline ($P < 0.05$). # significant overall difference between Bout 1 and Bout 2 ($P < 0.05$).

5.4 Discussion

In agreement with findings from Chapters 3 and 4, this study has demonstrated that an initial bout of resistance exercise decreased peak knee extensor strength and vertical jump performance, and increased perceived muscle soreness and CK response. Creatine kinase values were lower than those previously shown in studies that adopted similar modes of EIMD (Byrne & Eston 2002a; Davies et al. 2011a). However, this disparity might reflect an accelerated clearance of CK from the blood due to an increased blood flow (Warren et al. 1999) or that participants in this study had a better training status (Vincent & Vincent 1997). Notwithstanding this, impaired muscle function and increases in perceived muscle soreness suggest that EIMD occurred as a result of the first bout of squatting exercise.

The symptoms of EIMD that followed the first bout were attenuated when the same bout of resistance exercise was performed two weeks later. This attenuation is consistent with previous research (Byrnes et al., 1985; Newman et al., 1987; Nosaka & Clarkson, 1995; Hortobagyi et al., 1998; Howatson et al., 2007; Smith et al., 2007), and provides evidence that unaccustomed resistance exercise evoked an adaptation that enables the muscle to be more resistant to a repeated bout of the same exercise performed two weeks later. The justification for the 2-week interval between each bout of muscle damage was based on previous research. Using lower limb exercise to incur EIMD, research has shown that symptoms associated with EIMD are attenuated within 2 weeks (Clarkson & Tremblay, 1988; Eston et al., 1996; Hortobagyi et al., 1998; McHugh et al., 2001). Moreover, baseline markers of muscle soreness, knee extensor torque, vertical jump height and CK activity between Bout 1 and 2 were not significantly different ($P > 0.05$), thus providing support that the 2-week rest interval was sufficient for optimal recovery between bouts.

This study also observed that the physiological, metabolic, perceptual and kinematic responses during sub-maximal running were altered as a result of unaccustomed resistance exercise; the significant changes in $\dot{V}O_2$, \dot{V}_E , f_R , [La], RPE and lower limb kinematics after EIMD all concur with results from Chapters 3 and 4. However, this is the first study to demonstrate that the detrimental effects of EIMD on sub-maximal endurance exercise are attenuated after a second bout. Furthermore, this attenuation in sub-maximal endurance exercise after the repeated bout of muscle damage was similar to the attenuation shown in the indirect markers of muscle damage. Whilst the underlying mechanism responsible for the RBE is unknown, a reduced magnitude of muscle damage and its subsequent impact on sub-maximal running could be central or peripheral in origin, or a combination of both. Using surface EMG, several studies have observed that the frequency content (MF) of the EMG signal is decreased during a repeated bout of EIMD (Warren et al., 2000; Chen et al., 2003; Howatson et al., 2007; Starbuck & Eston, 2012), indicating a higher reliance on slow twitch muscle fibres (Warren et al., 2000). Given that slow twitch fibres are more resistant to muscle damage (Friden et al., 1983), it is plausible that an increased recruitment protects the muscle against a second bout of EIMD. Additionally, Kyrolainen et al. (2000) suggested that impaired muscle function following muscle-damaging exercise requires additional motor unit activation in order to produce the same resultant force during running at a given speed. As there is a linear relationship between EMG activity and oxygen cost (Bigland-Ritchie & Woods, 1974), the elevations in $\dot{V}O_2$ after the initial bout of squatting exercise (Bout 1) might have reflected such an increase in motor unit recruitment. However, with the $\dot{V}O_2$ response remaining unchanged during sub-maximal running after Bout 2, it is possible that a neural adaptation might have occurred. Indeed, an increased reliance

on slow twitch fibres during the repeated bout could have protected the muscle, causing muscle function to be unchanged in the days after Bout 2 and enabling normal motor unit recruitment to be maintained during the sub-maximal running. Nonetheless, future studies to confirm changes in neuromuscular recruitment during endurance exercise after EIMD are required.

Running uses the SSC to provide elastic energy during repeated eccentric and concentric actions (Komi, 2000). Prolonged exposure to resistance training improves running economy by increasing muscle stiffness regulation, allowing for more elastic energy to be stored during the eccentric phase (Bonacci et al., 2009). However, unaccustomed resistance exercise reduces muscle stiffness regulation and the subsequent ability to store elastic energy (Horita et al., 1996; 1999; Nicol et al., 1996). The reductions in drop jump performance provide indirect evidence that the force potential of the SSC was impaired after Bout 1. Therefore, an increase in $\dot{V}O_2$ during running could have been because of a reduced utilisation of elastic energy caused by the initial muscle-damaging exercise. That both drop jump performance and $\dot{V}O_2$ during sub-maximal running remained unchanged after the second bout of EIMD supports this.

The sensation of pain in skeletal muscle is believed to be signalled by Group III and IV fibres (Armstrong, 1984; Ebbeling & Clarkson, 1989; Jones & Round, 1990; Cleak & Eston, 1992a; Kendall & Eston, 2002). Indeed, it is hypothesized that the accumulation of noxious chemicals (such as prostaglandin, bradykinin, and histamine) activates afferent fibres to produce muscle soreness (Jones & Round, 1990). In attempts to limit the discomfort associated with muscle soreness, it is reasonable to assume that participants changed their running stride pattern, leading

to the observed increase in the oxygen cost of running. Indeed, the initial increase in $\dot{V}O_2$ occurred alongside decreases in stride length and increases in stride frequency during sub-maximal running, supporting the findings observed in Chapter 3. Accordingly, the attenuation in muscle soreness after the second bout would have enabled participants to maintain their normal stride pattern and explain why $\dot{V}O_2$ remained unchanged. The reduction in muscle soreness after Bout 2 also suggests that less stimulation was caused to Group III and IV afferent fibres.

Located in and around the skeletal muscle, Group III and IV afferent fibres are also responsible for modulating the ventilatory response to exercise (Haouzi et al., 2004) and are thought to be responsible for the increase in ventilation after eccentric exercise (Hotta et al., 2006; Davies et al., 2008; 2009; 2011a; Twist & Eston, 2009; Burt & Twist, 2011). Furthermore, muscle soreness also provides a stimulus to increase ventilatory response by activating nociceptive muscle afferents (Mense, 1977; 1982; Duranti et al., 1991). As anticipated there was an increase in \dot{V}_E and f_R after the first bout of squatting exercise that was consistent with findings shown in Chapters 3 and 4. However, ventilatory responses remained unchanged after the second bout. These findings are likely to be explained by the repeated bout effect; namely the lower magnitude of muscle damage and reductions in perceived muscle soreness after the repeated bout of squatting exercise. Indeed, the maintenance of muscle fibre integrity (evidenced by lower CK and preservation of force after Bout 2) would not result in the increased discharge from afferent fibres to increase ventilation as described by Hotta et al. (2006). Similarly, the lower perceptions of muscle pain after Bout 2 would not have the same stimulating effect on ventilation that was perhaps observed after the initial bout.

An increased reliance on non-damaged type II muscle fibres is thought to be responsible for elevations in [La] that accounts for the increased ventilatory response observed whilst exercising with muscle damage (Gleeson et al., 1995; Braun & Dutto, 2003). The \dot{V}_E response is increased to excrete the additional CO_2 produced because of elevated [La]. In this study [La] was greater during running after Bout 1 than after Bout 2, suggesting that the attenuated \dot{V}_E response after Bout 2 was due to a reduction in [La]. However, as $\dot{V}_E/\dot{V}CO_2$ was greater during running after Bout 1 than Bout 2, indicating that \dot{V}_E was not associated with changes in CO_2 , it is unlikely that changes in [La] was responsible for the attenuated \dot{V}_E response after Bout 2.

The elevation of RPE during sub-maximal running exercise after the first bout of muscle-damaging exercise is consistent with the findings from Chapters 3 and 4. Hampson et al. (2001) proposed that perceived exertion involves a combination of sensory cues derived from a number of different factors. Indeed, Jameson and Ring (2000) suggest that muscle pain and feelings of breathlessness are determinants of RPE during endurance exercise. Likewise, Scott et al. (2003) and Elmer et al. (2010) attribute a greater perceived exertion to the increased motor unit recruitment required to produce the same baseline sub-maximal force after muscle-damaging exercise. If these responses provided cues for RPE in the current study, it is likely that their attenuation after Bout 2 explains the unchanged RPE response. Moreover, it is possible that the same neural and/or peripheral adaptations highlighted above to explain the reduction in muscle soreness, ventilatory response and neuromuscular function contribute to the attenuation in RPE response.

Whilst this is the first study to demonstrate that the RBE is transferable to endurance performance, the findings are limited to fixed intensity endurance running. Such

protocols allow the physiological, metabolic and perceptual responses to be monitored during steady state endurance exercise; nonetheless, they do not replicate real-world endurance events. Alternatively, time-trials, whereby individuals cover a set distance in the quickest time possible, are a more realistic marker of endurance performance. Therefore, one of the aims of the next study will be to investigate the effects of the RBE on running time-trial performance.

In conclusion, findings from Chapters 3 and 4 suggest that EIMD has a negative impact on endurance exercise performed in the days that follow. However, it was not known whether such effects occur after a repeated bout of EIMD. Therefore, the aim of this study was to investigate the effects of repeated bouts of muscle-damaging exercise on sub-maximal running exercise. To address this aim, participants completed a bout of sub-maximal running before, 24 and 48 h after an initial bout of muscle-damaging squatting exercise. Two weeks later, participants repeated the same baseline procedures, muscle-damaging exercise and follow-up testing. In agreement with Chapters 3 and 4, this investigation reaffirms that the physiological, perceptual, and kinematic responses during sub-maximal running are altered in the days following unaccustomed resistance exercise. However, following this initial bout of muscle-damaging exercise, an adaptation occurs to the muscle, which is shown to attenuate the detrimental effects of muscle damage on the physiological, metabolic, perceptual and kinematic responses during sub-maximal running. It is unlikely that any one mechanism explains the observed RBE; rather it is probable that the interaction of various neural and peripheral adaptations occurred to protect the muscle against the detrimental effects of EIMD during sub-maximal running exercise. Importantly, these findings have applied implications for both endurance athletes and recreationally active populations wishing to embark on concurrent training for the first

time. In the first instance, such people need to be aware of the negative effects that unaccustomed resistance exercise can have on the muscle and how this impacts upon further exercise performed in the days following. Thereafter, as the current study has demonstrated, less recovery will be required in the days after a repeated bout of resistance exercise performed in the few weeks that follow. What remains to be investigated is if a bout of low volume muscle-damaging exercise performed two weeks prior to a bout of high volume EIMD provides the same protective effect on endurance running exercise. Furthermore, prior research has demonstrated that EIMD reduces endurance time-trial performance; nevertheless, it remains to be seen if this reduction is still evident as a result of the RBE.

CHAPTER 6

A LOW VOLUME OF MUSCLE-DAMAGING EXERCISE CONFERS PROTECTION AGAINST A HIGH VOLUME OF MUSCLE-DAMAGING EXERCISE AND THE DETRIMENTAL EFFECTS ON ENDURANCE RUNNING

6.1 Introduction

Findings from the previous three chapters clearly demonstrate that EIMD increases oxygen consumption ($\dot{V}O_2$) during fixed-intensity running. One of the mechanisms posited to explain this rise in $\dot{V}O_2$ is an increase in motor unit recruitment (Kyrolainen et al., 2000; Braun & Dutto, 2003; Chen et al., 2007b). Kyrolainen et al. (2000) postulated additional motor units are recruited in order to attain the same force during fixed-intensity running when the muscle is damaged. However, it has not been confirmed if motor unit activation is increased during running as a result of muscle damage. Therefore, an investigation incorporating EMG would confirm if increases in motor unit recruitment occur alongside increases in $\dot{V}O_2$ during fixed-intensity running after EIMD.

It is also well established that the signs and symptoms of EIMD are attenuated after a repeated bout of muscle-damaging exercise (Byrnes et al., 1985; Newman et al., 1987; Nosaka & Clarkson, 1995; McHugh et al., 1999a; McHugh, 2003). These adaptations are now known to be extended to the observed effects of EIMD on running performance (see Chapters 3, 4 and 5), whereby the alterations in the physiological, metabolic, perceptual and kinematic responses during fixed-intensity running are attenuated after a repeated bout of EIMD performed two weeks later (see Chapter 5).

An interesting observation is that a prior bout of low volume eccentric exercise protects the muscle against damage in the days after high volume muscle-damaging exercise (Schwane & Armstrong, 1983; Clarkson & Tremblay, 1988; Brown et al., 1997; Nosaka et al., 2001b; Chen, 2003; Chen & Nosaka, 2006). Howatson et al. (2007) demonstrated that 10 maximal eccentric contractions protected the elbow

flexors against 45 maximal eccentric contractions performed two weeks later. Here the RBE was attributed to a neural adaptation that facilitated a greater activation of more robust type I muscle fibres during the high volume eccentric exercise. However, it is not known whether the protective effect of low volume muscle-damaging exercise performed prior to a high volume of the same exercise preserves running performance (as observed in Chapter 5). Such a study would have 'real-world' application for those endurance athletes engaging in periodized resistance training for the first time. That is to say where endurance athletes are contemplating concurrent endurance and resistance exercise to improve performance, performing a low volume of resistance exercise two weeks before engaging in concurrent training might precondition the muscle to withstand higher bouts of muscle-damaging exercise and its effects on endurance performance.

The previous chapters of this thesis have examined the effects of EIMD on fixed-intensity endurance exercise; whilst such protocols enable physiological responses to be measured during steady state exercise, they do lack ecological validity (Currell & Jeukendrup, 2008). Alternatively, time-trials provide a more accurate measurement of endurance performance and research has shown time-trial performance is impaired as a result of EIMD. Indeed, decreases in distance covered (~6%), power output (~12%) and oxygen cost (~11%) have all been observed during cycling and running time-trial performances after EIMD (Marcora & Bosio, 2007; Twist & Eston, 2009; Burt & Twist, 2011). The mechanisms to explain the decrement in time-trial performance include an altered sense of effort, a reduction in neural drive and an increased production of inflammatory cytokines (Marcora & Bosio, 2007; Twist & Eston, 2009; Burt & Twist, 2011). However, it is unknown if this reduction in time-trial performance is still evident after a repeated bout of EIMD. This

study aimed to 1) investigate if increases in motor unit recruitment occur alongside increases in $\dot{V}O_2$ during fixed-intensity running after EIMD; 2) to reaffirm findings from study 3; that changes to $\dot{V}O_2$, \dot{V}_E , [La], RPE, SL and SF during sub-maximal running after initial EIMD are reduced in the days after a repeated bout of EIMD; 3) to confirm that a prior bout of low volume (50 squats) muscle-damaging exercise protects the muscle against symptoms of EIMD following high volume (100 squats) muscle-damaging exercise; 4) to investigate whether a low volume of muscle-damaging exercise can protect the muscle against the detrimental effects on $\dot{V}O_2$, \dot{V}_E , [La], RPE, SL and SF responses during fixed-intensity running after a subsequent bout of high volume EIMD; 5) to confirm the detrimental effects of EIMD on running time-trial performance; focusing on whether time, speed, $\dot{V}O_2$, HR and [La] are changed during time-trial running as a result of EIMD; 6) to examine whether the negative implications of EIMD on time-trial running are negated following a repeated exposure to muscle-damaging exercise.

6.2 Methods

6.2.1 Participants

After Institutional ethical approval was granted from the Faculty of Applied Sciences Research Ethics Committee, 16 healthy male participants, who had not completed any form of lower limb resistance exercise in the previous six months, volunteered for the study. Prior to data collection, participants provided informed consent and completed a medical health questionnaire. Participant characteristics are shown below (see Table 6.1).

Table 6.1 Participant characteristics

	Low-High (n = 8)	High-High (n = 8)
Age (y)	26 ± 5	27 ± 4
Stature (m)	1.79 ± 0.05	1.77 ± 0.1
Body Mass (kg)	79.5 ± 7.8	76.7 ± 9.8
$\dot{V}O_{2peak}$ (ml·kg ⁻¹ ·min ⁻¹)	54.8 ± 3.5	54.2 ± 4.1
HR _{peak} (b·min ⁻¹)	191 ± 10	191 ± 9
LTP _{speed} (km·h ⁻¹)	12.4 ± 1.1	12.6 ± 0.9

Abbreviations: $\dot{V}O_{2peak}$ = peak oxygen uptake; HR_{peak} = peak heart rate at peak oxygen uptake; LTP_{speed} = speed corresponding to lactate turnpoint.

6.2.2 Study Design

Participants were tested on eight separate occasions over a 5-week period (see Figure 6.1). During visit 1, participants completed an incremental exhaustive running test to determine LTP and $\dot{V}O_{2peak}$. This was followed by habituation procedures for perceived muscle soreness, peak knee extensor torque (isometric and isokinetic) and 3 km running time-trial. After 48 – 72 h, participants completed isometric maximal voluntary contractions (MVCs) of the knee extensors, during which EMG activity of the vastus medialis (VM) and vastus lateralis (VL) was recorded (visit 2). Upon completion, participants were randomly assigned to either a low volume (n = 8) or high volume (n = 8) muscle damage group. After a further 48 – 72 h, participants completed pre-damage perceived muscle soreness, isokinetic peak knee extensor torque, CK activity, fixed-intensity running, a 3 km running time-trial and either a low or high volume bout of lower limb resistance exercise designed to develop symptoms of EIMD (visit 3). Baseline procedures were then repeated 24 and 48 h later with the exception of the time-trial at 24 h (visit 4 and 5). Two weeks later, when symptoms associated with the initial bout of muscle damage had

disappeared (Hortobagyi et al., 1998), participants repeated baseline procedures, a repeated bout of high volume muscle-damaging exercise (visit 6) and the same follow-up testing at 24 and 48 h (except for the time-trial at 24 h) (visit 7 and 8). Participants were requested to avoid exercise 24 h prior to each visit, maintain their normal diet and avoid using any analgesic agents.

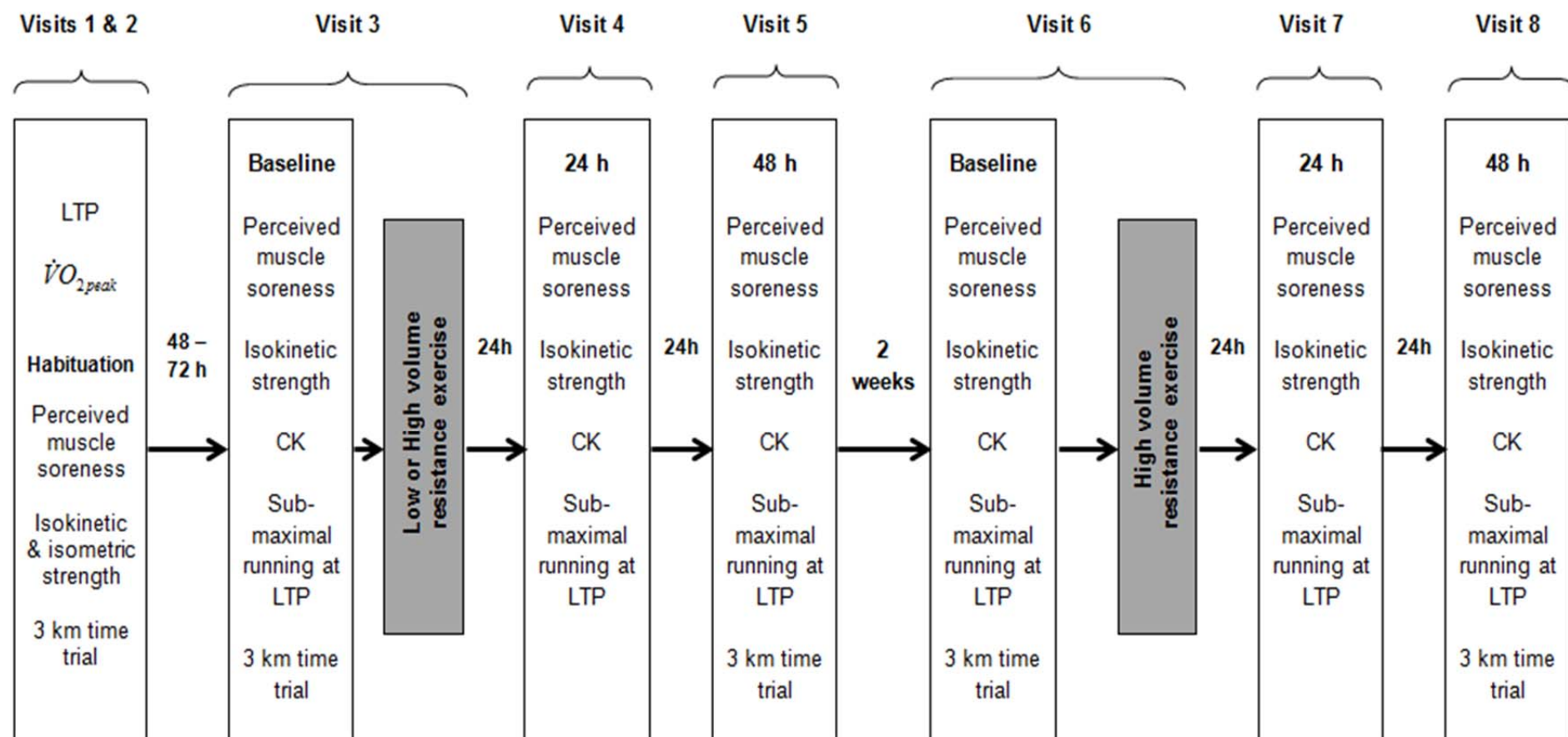


Figure 6.1. Schematic of the study design

6.2.3 Assessment of lactate turnpoint and peak oxygen uptake

Incremental running to determine individual LTP and $\dot{V}O_{2peak}$ was performed using the methods outlined in Chapter 4.

6.2.4 Perceived muscle soreness

Participants indicated the perceived soreness felt in their knee extensors using the VAS scale detailed in Chapter 3.

6.2.5 Assessment of peak isometric and isokinetic knee extensor torque

To normalise peak EMG amplitude, recorded during each fixed-intensity running bout, EMG activity of the VM and VL was recorded during isometric MVCs of the knee extensors (Ansley et al., 2004) measured from the dominant limb using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). Peak isometric force was assessed at an angle of 80°, with full knee extension providing a 0° reference (Byrne et al., 2001). Before the test, participants performed a standardized warm-up comprising 3 min of sub-maximal cycling at 50 W (Monark, 874E, Monark, Varberg, Sweden). After five sub-maximal and one maximal familiarization trials, participants performed two MVCs of 3 s duration with a 60 s rest between each trial (Byrne et al., 2001).

To assess muscle function after the initial and repeated bouts of EIMD, isokinetic peak knee extensor torque was determined from the dominant limb using the same procedures described in Chapter 3.

6.2.6 Assessment of creatine kinase

Plasma blood CK activity was assessed using the same method described in Chapter 3.

6.2.7 Fixed-intensity running and stride pattern

Participants performed 5 min of fixed-intensity running at their previously determined LTP on a motorized treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany). Expired air, HR, RPE and [La] were recorded as previously described in Chapter 3. The final 10 s of each 5-min running bout was recorded, downloaded and digitized using motion analysis software (Quintec Biomechanics 9.03 v 14). Stride length and SF during each running bout were determined from the methods previously described in Chapter 3.

6.2.8 Electromyography

During the isometric MVCs and each fixed-intensity running bout, surface EMG (Noraxon, Scottsdale, AZ, USA) was recorded from the VM and VL. The location of each muscle site was in accordance with the Surface EMG for Non-Invasive Assessment of Muscles (SENIAM) recommendations (Hermens et al., 1999). Briefly, electrodes for the VM were placed at four-fifths of the distance between the anterior superior iliac spine and the joint space in front of the anterior border of the medial ligament. The VL electrode site was located at two-thirds of the distance from the anterior superior iliac spine and the lateral side of the patella. A ground reference electrode was placed over the tibia. Before the placement of electrodes, body hair was removed, the skin lightly abraded and cleansed with an alcohol swab. In agreement with recommendations from SENIAM, dual silver/silver chloride

electrodes (10 mm diameter contact area, 20 mm inter-electrode distance and 40 mm x 22 mm adhesive gel surface) were aligned with the orientation of the muscle fibre. Once in place, the electrode-skin impedance was checked to ensure it was < 10 K Ω . Electrodes were connected, via wires with built-in pre-amplifiers, to a transmitter unit that relayed data to an analogue-to-digital converter through telemetry. The wires and pre-amplifiers were taped to the skin to prevent any movement artefact. The raw EMG signals were sampled at a frequency of 1500 Hz, amplified by 500 and underwent a 12-bit analog-to-digital conversion. The common mode rejection ratio was > 100 dB, the baseline noise < 2 μ V and the input impedance > 10 M Ω (Konrad, 2005). Data were collected during all of the 5-min fixed-intensity running bouts, with the last 10 contractions analysed for peak EMG amplitude and median frequency (MF). To calculate the peak EMG amplitude, the raw data were full-wave rectified using root mean squared averaging with a 50 ms time window and then filtered with a band-pass of 10 – 500 Hz (Konrad, 2005). All peak EMG amplitude data was normalized by dividing the value during each fixed-intensity running bout by the peak EMG amplitude obtained during the peak isometric MVC (Ansley et al., 2004).

To generate the MF, the sampled raw EMG signal was processed using a fast Fourier transform (FFT) to create a power density spectrum. The MF was calculated as the point at which the power spectrum was divided into equal halves of low and high-frequency (Kamen & Caldwell, 1996). After the first trial, the position of each electrode was marked with permanent ink to ensure the same placement across the bouts.

6.2.9 Three kilometre time-trial protocol

Participants were required to complete 3 km in the quickest time possible on a motorized treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany) set at a 1% incline (Jones & Doust, 1996). Feedback on distance covered was freely available, however participants were not able to view time, treadmill speed or heart rate. As previously described by Marcora and Bosio (2007), the time-trial commenced with the participants standing on the treadmill whilst the speed was increased to 9 km·h⁻¹. Once this speed was attained, participants were able to adjust the speed freely. Time and a fingertip capillary sample analysed for [La] were collected upon completion of the time-trial. Expired air and heart rate were measured throughout the time trial, with mean $\dot{V}O_2$ and heart rate determined for analysis. Rating of perceived exertion was recorded in the last 50 m of the time-trial. Average speed was calculated from dividing distance by time.

6.2.10 Exercise-induced muscle damage protocol

The same muscle-damaging protocol as detailed in Chapter 3 was performed by participants in this current study. The low volume group completed an initial bout of resistance exercise consisting of 5 sets of 10 squats, followed two weeks later by a higher volume bout of 10 sets of 10 squats. The high volume group completed an initial bout of 10 sets of 10 squats, followed two weeks later by repeating the same high volume bout.

6.2.11 Statistical analysis

Descriptive statistics (mean ± SD) were calculated for all performance variables. To test for differences between the indirect markers of EIMD and dependent variables

during the fixed-intensity running protocol a series of separate three-way mixed factor (Group [2] and Bout [2] and Time [3]) repeated measures analyses of variance (ANOVAs) were conducted. Dependant variables during the time-trial were also analysed using a mixed factor repeated measures ANOVA (Group [2] and Bout [2] and Time [2]). Assumptions of sphericity were assessed using Mauchly's test, with any violations adjusted by use of the Greenhouse-Geisser correction. Where significant group x bout x time interaction effects were observed, independent *t*-tests were used to identify differences. The alpha level was set at $P < 0.05$.

6.3 Results

6.3.1 Indirect markers of muscle damage

Perceived muscle soreness demonstrated a significant Bout ($F_{(1,14)} = 33.9$, $P \leq 0.0005$) and Bout x Time ($F_{(2,28)} = 24.3$, $P \leq 0.0005$) effect, however, no significant Group x Bout x Time interaction was observed ($F_{(2,28)} = 1.3$, $P = 0.29$). There was no Group x Bout x Time interaction for isokinetic peak knee extensor torque ($F_{GG(1.2,16.2)} = 1.3$, $P = 0.296$), although a Bout ($F_{(1,14)} = 15.4$, $P = 0.002$) and a Bout x Time ($F_{GG(1.2,16.2)} = 11.6$, $P = 0.003$) effect was shown. CK activity changed over Bout ($F_{(1,14)} = 28.2$, $P \leq 0.0005$) and across Bout x Time ($F_{GG(1.2,17.3)} = 37.8$, $P \leq 0.0005$). A Group x Bout x Time interaction ($F_{GG(1.2,17.3)} = 7.9$, $P = 0.009$) was also observed, with *post hoc* analysis revealing that CK activity was significantly greater in the High-High group at 24 and 48 h after Bout 1 (Figure 6.2).

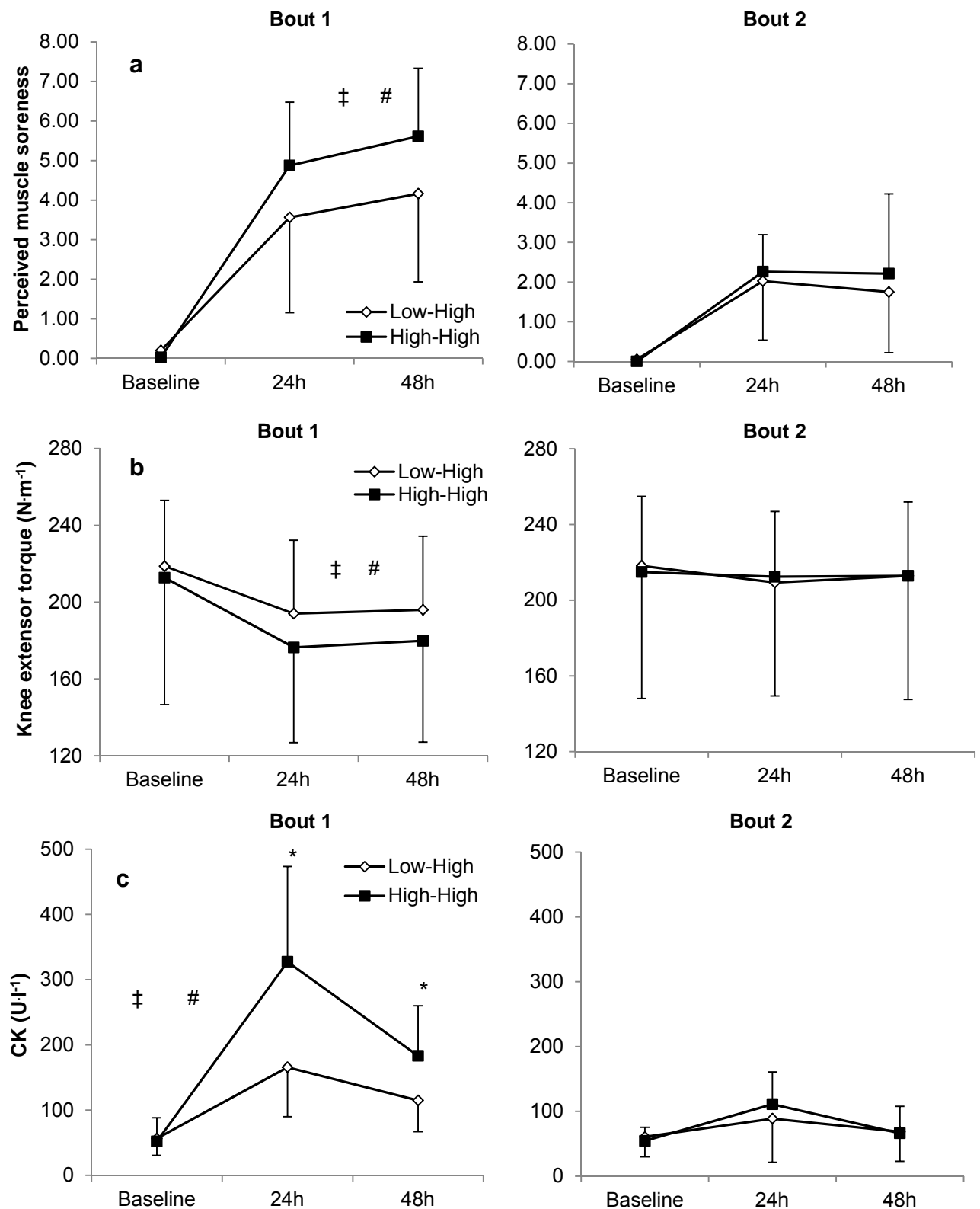


Figure 6.2. Changes in **a** perceived muscle soreness, **b** knee extensor torque and **c** CK after repeated bouts of muscle-damaging exercise. Values are shown as means \pm SD. ‡ denotes a significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # denotes a significant Bout \times Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$). * denotes a Group \times Bout \times Time interaction; High-High group > Low-High Group at 24 – 48 h after Bout 1 ($P < 0.05$).

6.3.2 Fixed-intensity running responses to repeated bouts of muscle-damaging exercise

Most of the physiological, metabolic and perceptual responses during fixed-intensity running revealed significant Bout and Bout x Time effects. Heart rate demonstrated a Bout effect, but no significant Bout x Time interaction was observed. There was no significant interaction of Group x Bout x Time on $\dot{V}O_2$, \dot{V}_E , [La], RPE, HR, f_R , $\dot{V}_E/\dot{V}O_2$ or $\dot{V}_E/\dot{V}CO_2$ (Table 6.2; results are shown in Table 6.4).

Table 6.2 Three-way ANOVA results during fixed-intensity running after repeated bouts of EIMD

Variable	Bout	Bout x Time	Group x Bout x Time
$\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	$F_{(1,14)} = 30.2, P \leq 0.0005$	$F_{(2,28)} = 16.1, P \leq 0.0005$	$F_{(2,28)} = 0.7, P = 0.495$
\dot{V}_E (l·min ⁻¹)	$F_{(1,14)} = 9.4, P = 0.008$	$F_{(2,28)} = 18.2, P \leq 0.0005$	$F_{(2,28)} = 0.4, P = 0.684$
[La] (mmol·l ⁻¹)	$F_{(1,14)} = 25.4, P \leq 0.0005$	$F_{(2,28)} = 5.9, P = 0.007$	$F_{(2,28)} = 2.6, P = 0.096$
RPE	$F_{(1,14)} = 20.1, P = 0.001$	$F_{(2,28)} = 25.6, P \leq 0.0005$	$F_{(2,28)} = 0.5, P = 0.642$
HR (b·min ⁻¹)	$F_{(1,14)} = 7.1, P = 0.019$	$F_{(2,28)} = 1.1, P = 0.359$	$F_{(2,28)} = 0.5, P = 0.595$
f_R (breaths·min ⁻¹)	$F_{(1,14)} = 8.3, P = 0.012$	$F_{(2,28)} = 12.5, P \leq 0.0005$	$F_{(2,28)} = 1.1, P = 0.336$
$\dot{V}_E/\dot{V}O_2$	$F_{(1,14)} = 4.7, P = 0.049$	$F_{GG(1.4,19.3)} = 5.1, P = 0.026$	$F_{GG(1.4,19.3)} = 0.4, P = 0.629$
$\dot{V}_E/\dot{V}CO_2$	$F_{(1,14)} = 5.5, P = 0.035$	$F_{(2,28)} = 13.0, P \leq 0.0005$	$F_{(2,28)} = 1.2, P = 0.316$

6.3.3 Stride pattern

Running stride pattern demonstrated significant Bout (SL ($F_{(1,14)} = 34.2, P \leq 0.0005$); SF ($F_{(1,14)} = 36.5, P \leq 0.0005$)) and Bout x Time (SL ($F_{(2,28)} = 13.7, P \leq 0.0005$); SF ($F_{(2,28)} = 15.2, P \leq 0.0005$) effects. However, there was no significant difference between the Low-High and High-High groups (Group x Bout x Time: SL ($F_{(2,28)} = 0.8, P = 0.469$); SF ($F_{(2,28)} = 0.5, P = 0.597$)) (Table 6.5).

6.3.4 Electromyography

Median frequency during fixed-intensity running for the VM (Bout ($F_{(1,14)} = 0.2$, $P = 0.648$); Bout x Time ($F_{(2,28)} = 0.5$, $P = 0.609$); Group x Bout x Time ($F_{(2,28)} = 0.4$, $P = 0.685$) and VL (Bout ($F_{(1,14)} = 0.8$, $P = 0.400$); Bout x Time ($F_{(2,28)} = 1.6$, $P = 0.230$); Group x Bout x Time ($F_{(2,28)} = 1.0$, $P = 0.370$) was not significantly different after repeated bouts of muscle-damaging exercise. Peak EMG amplitude of the VM demonstrated a significant main effect of Bout ($F_{(1,14)} = 17.5$, $P = 0.001$) and an interaction effect of Bout x Time ($F_{(2,28)} = 9.8$, $P = 0.001$). However, there was no significant difference between the groups (Group x Bout x Time: $F_{(2,28)} = 1.1$, $P = 0.342$). Peak EMG amplitude of the VL demonstrated a significant Bout effect ($F_{(1,14)} = 27.4$, $P \leq 0.0005$), however, there was no interaction effect of Bout x Time ($F_{(2,28)} = 2.2$, $P = 0.130$) or Group x Bout x Time ($F_{(2,28)} = 0.4$, $P = 0.665$) (Table 6.6).

6.3.5 Time trial responses to repeated bouts of muscle-damaging exercise

Analysis revealed a significant main effect of Bout and an interaction effect of Bout x Time on most variables presented. However, there was no Bout or Bout x Time effect for end RPE response during the time-trial. Furthermore, there was no significant interaction of Group x Bout x Time on time, average speed, mean $\dot{V}O_2$, end [La], mean HR or end RPE (Table 6.3; results are shown in Figure 6.3).

Table 6.3 Three-way ANOVA results during time trial running after repeated bouts of EIMD

Variable	Bout	Bout x Time	Group x Bout x Time
Time (s)	$F_{(1,14)} = 39.3, P \leq 0.0005$	$F_{(1,14)} = 29.7, P \leq 0.0005$	$F_{(1,14)} = 0.3, P = 0.614$
Speed (km·h ⁻¹)	$F_{(1,14)} = 29.9, P = \leq 0.0005$	$F_{(1,14)} = 25.5, P \leq 0.0005$	$F_{(1,14)} = 0.9, P = 0.364$
$\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	$F_{(1,14)} = 15.9, P = 0.001$	$F_{(1,14)} = 19.1, P = 0.001$	$F_{(1,14)} = 2.2, P = 0.163$
[La] (mmol·l ⁻¹)	$F_{(1,14)} = 33.8, P \leq 0.0005$	$F_{(1,14)} = 15.2, P = 0.002$	$F_{(1,14)} = 0.01, P = 0.938$
HR (b·min ⁻¹)	$F_{(1,14)} = 7.2, P = 0.018$	$F_{(1,14)} = 21.1, P \leq 0.0005$	$F_{(1,14)} = 3.9, P = 0.07$
RPE	$F_{(1,14)} = 0.7, P = 0.425$	$F_{(1,14)} = 1.1, P = 0.312$	$F_{(1,14)} = 0.3, P = 0.609$

Table 6.4 Mean (\pm SD) physiological, metabolic and perceptual responses during fixed-intensity running after repeated bouts of muscle-damaging exercise

Variable	Bout	Baseline	Low-High		Baseline	High-High	
			24	48 h		24 h	48 h
$\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹) ‡ #	1	46.9 \pm 2.3	48.7 \pm 3.1	48.9 \pm 3.0	47.3 \pm 2.8	49.5 \pm 2.7	49.9 \pm 3.1
	2	46.7 \pm 2.4	47.1 \pm 2.5	46.9 \pm 2.7	47.5 \pm 2.8	47.4 \pm 3.1	47.4 \pm 2.8
\dot{V}_E (l·min ⁻¹) ‡ #	1	101.3 \pm 11.7	110.8 \pm 12.4	109.3 \pm 12.4	101.3 \pm 21.9	112.4 \pm 26.5	114.3 \pm 26.3
	2	102.6 \pm 11.8	104.4 \pm 10.9	103.3 \pm 11.9	100.3 \pm 16.3	102.9 \pm 15.6	103.1 \pm 18.4
[La] (mmol·l ⁻¹) ‡ #	1	4.8 \pm 1.0	5.5 \pm 1.2	5.1 \pm 1.2	5.7 \pm 1.5	6.1 \pm 1.4	6.6 \pm 1.8
	2	4.6 \pm 1.1	4.8 \pm 1.1	4.6 \pm 1.0	5.6 \pm 1.6	5.7 \pm 1.6	5.7 \pm 1.4
RPE ‡ #	1	12.6 \pm 0.9	14.1 \pm 1.1	13.8 \pm 1.0	13.0 \pm 1.5	14.8 \pm 1.6	14.3 \pm 1.6
	2	12.6 \pm 0.7	12.9 \pm 1.1	12.6 \pm 0.7	13.3 \pm 1.2	13.5 \pm 1.3	13.5 \pm 1.3
HR (b·min ⁻¹) ‡	1	163.4 \pm 11.6	166.4 \pm 11.5	165.8 \pm 11.2	172.5 \pm 10.2	172.9 \pm 11.2	173.5 \pm 9.5
	2	163.3 \pm 11.4	164.5 \pm 10.1	163.9 \pm 10.0	170.8 \pm 11.0	171.0 \pm 11.6	171.3 \pm 11.1
f_R (breaths·min ⁻¹) ‡ #	1	36.6 \pm 7.5	40.9 \pm 7.5	40.9 \pm 8.2	36.9 \pm 4.2	42.3 \pm 6.1	42.9 \pm 6.4
	2	36.4 \pm 6.8	37.6 \pm 7.2	38.4 \pm 7.3	36.8 \pm 3.7	38.5 \pm 3.5	38.3 \pm 4.1
$\dot{V}_E / \dot{V}O_2$ ‡ #	1	27.3 \pm 3.6	28.9 \pm 3.8	28.3 \pm 3.4	27.9 \pm 2.4	29.5 \pm 3.1	29.8 \pm 3.4
	2	27.9 \pm 3.3	28.2 \pm 3.3	27.9 \pm 3.4	27.6 \pm 1.9	28.4 \pm 2.3	28.4 \pm 2.6
$\dot{V}_E / \dot{V}CO_2$ ‡ #	1	27.7 \pm 3.6	29.3 \pm 3.8	29.3 \pm 4.1	27.3 \pm 2.0	29.8 \pm 2.5	29.8 \pm 3.2
	2	28.3 \pm 3.2	28.7 \pm 3.3	28.0 \pm 3.3	27.4 \pm 1.7	28.6 \pm 1.6	28.8 \pm 2.6

‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$).

Table 6.5 Mean (\pm SD) stride length and frequency responses during fixed-intensity running after repeated bouts of muscle-damaging exercise

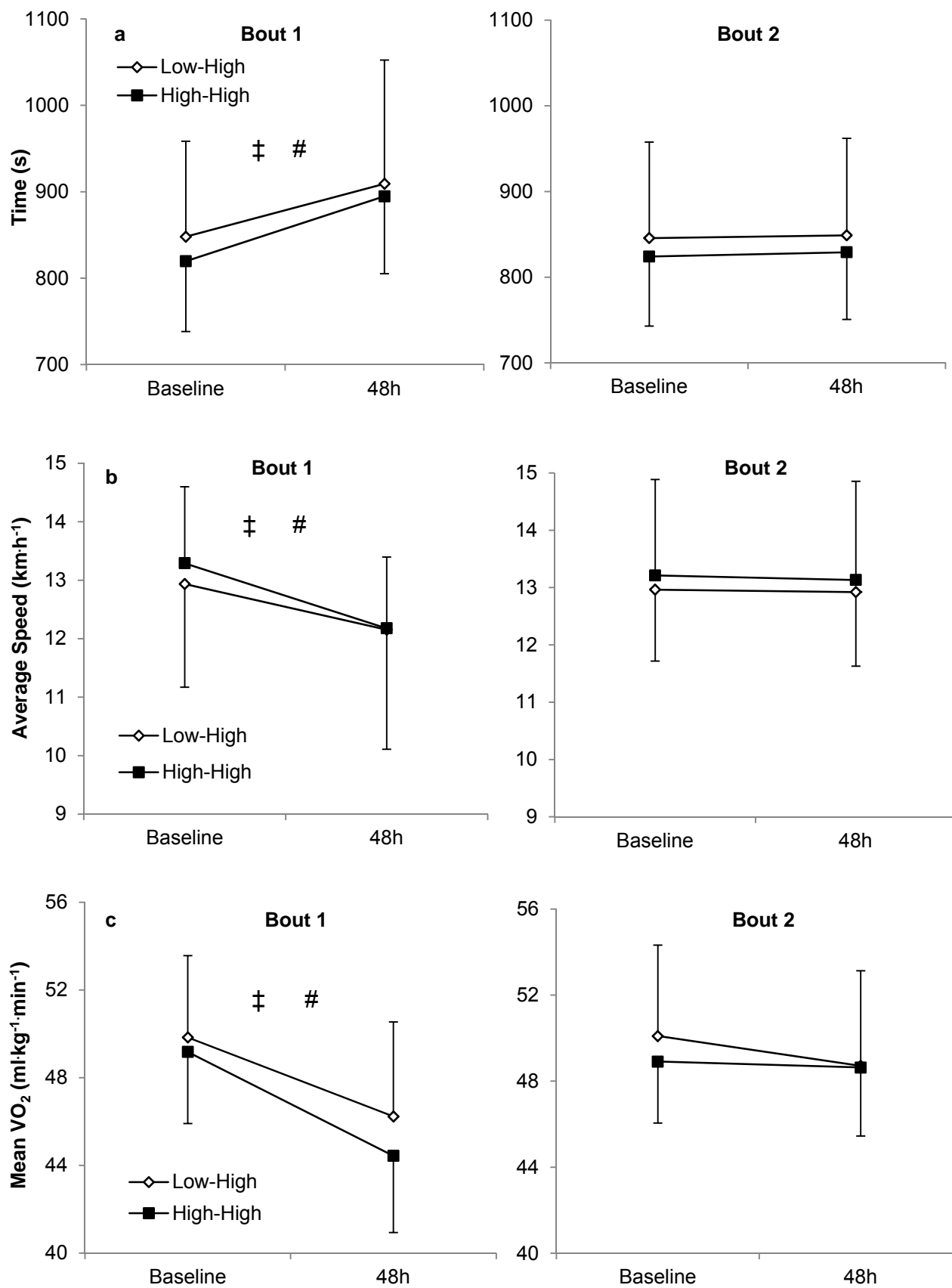
Variable	Bout	Low-High			High-High		
		Baseline	24 h	48 h	Baseline	24 h	48 h
SL (m^{-1}) ‡ #	1	2.39 \pm 0.17	2.33 \pm 0.19	2.33 \pm 0.18	2.43 \pm 0.20	2.35 \pm 0.19	2.33 \pm 0.18
	2	2.41 \pm 0.18	2.40 \pm 0.18	2.40 \pm 0.18	2.45 \pm 0.21	2.44 \pm 0.20	2.43 \pm 0.20
SF ‡ #	1	14.4 \pm 1.1	14.8 \pm 1.2	14.8 \pm 1.2	14.5 \pm 0.8	14.9 \pm 0.7	15.1 \pm 0.7
	2	14.3 \pm 1.1	14.3 \pm 1.1	14.4 \pm 1.1	14.4 \pm 0.8	14.4 \pm 0.8	14.5 \pm 0.8

‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$).

Table 6.6 Mean (\pm SD) median frequency and peak EMG amplitude responses during fixed-intensity running after repeated bouts of muscle-damaging exercise

Variable	Bout	Low-High			High-High		
		Baseline	24 h	48 h	Baseline	24 h	48 h
MF VM (Hz)	1	66.7 \pm 15.8	65.0 \pm 14.3	64.4 \pm 15.0	72.3 \pm 14.5	66.2 \pm 10.7	69.1 \pm 11.1
	2	66.0 \pm 12.2	64.0 \pm 11.1	66.2 \pm 11.4	69.0 \pm 9.5	65.9 \pm 11.9	67.8 \pm 10.5
MF VL (Hz)	1	66.1 \pm 9.8	62.0 \pm 6.2	67.4 \pm 10.1	70.6 \pm 9.4	68.0 \pm 12.2	69.4 \pm 11.6
	2	63.9 \pm 9.3	65.8 \pm 7.3	63.0 \pm 8.2	68.6 \pm 11.8	67.6 \pm 7.1	68.5 \pm 9.4
EMG Peak VM (%) ‡ #	1	66.8 \pm 22.3	72.5 \pm 24.4	71.0 \pm 22.3	49.3 \pm 14.7	62.6 \pm 15.0	59.8 \pm 19.4
	2	66.2 \pm 21.9	63.3 \pm 21.5	65.5 \pm 23.0	50.2 \pm 20.7	48.4 \pm 18.0	48.1 \pm 17.8
EMG Peak VL (%) ‡	1	68.8 \pm 17.3	72.4 \pm 14.7	71.4 \pm 11.6	52.0 \pm 13.0	57.0 \pm 17.0	56.7 \pm 14.4
	2	61.8 \pm 16.1	62.0 \pm 14.7	61.5 \pm 13.8	51.8 \pm 15.5	51.2 \pm 7.7	48.6 \pm 11.1

‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$). *Abbreviations:* MF VM = median frequency for vastus medialis, MF VL = median frequency for vastus lateralis, EMG Peak VM = Peak electromyography amplitude for vastus medialis, EMG Peak VL = Peak electromyography amplitude for vastus lateralis.



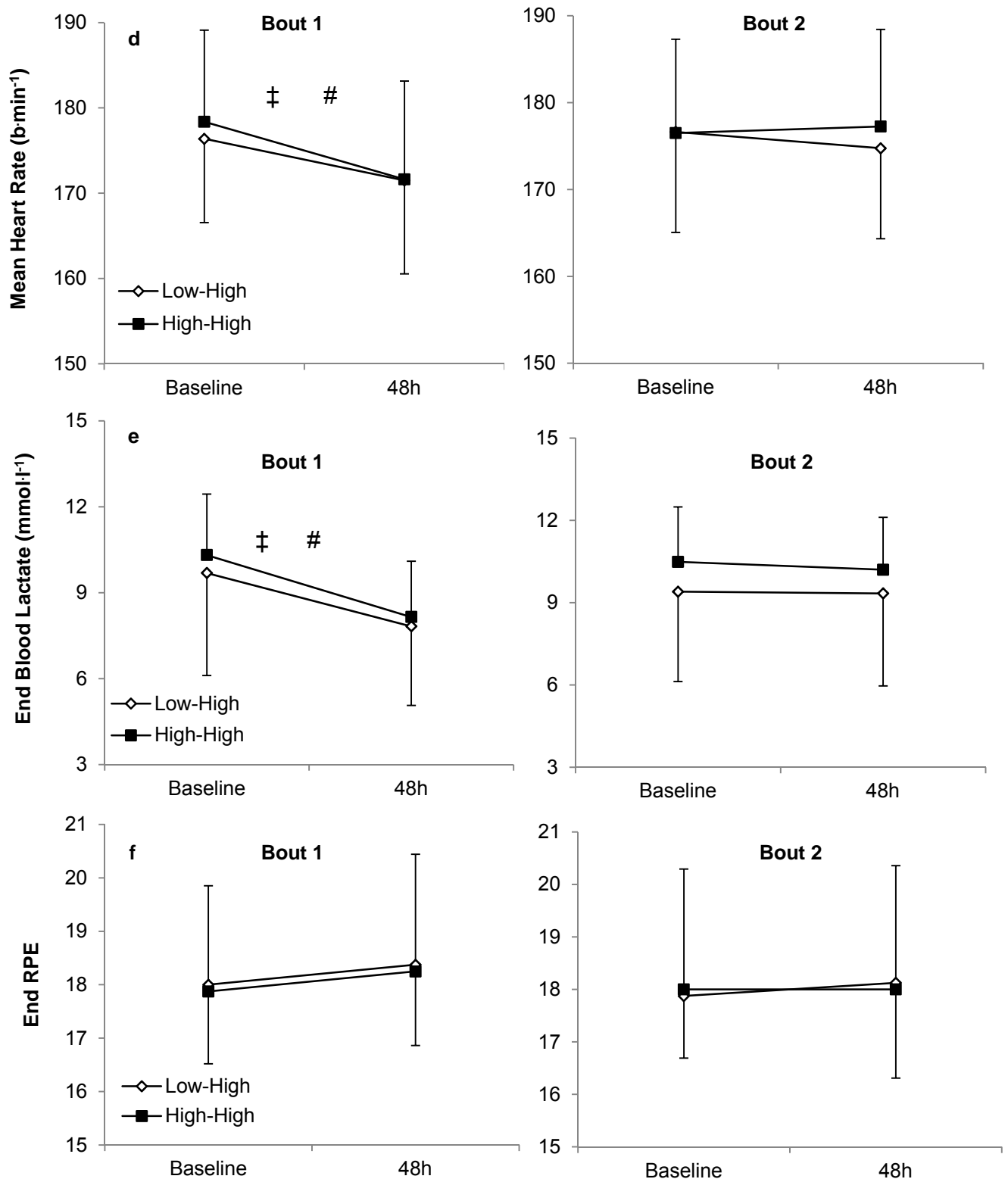


Figure 6.3. Changes in **a** time, **b** average speed, **c** mean $\dot{V}O_2$, **d** mean heart rate, **e** end blood lactate and **f** end RPE during time-trial running after repeated bouts of EIMD. Values are shown as means \pm SD. ‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$).

6.4 Discussion

The initial bout of squatting exercise, independent of volume, resulted in symptoms of muscle damage. Both groups experienced heightened perceptions of muscle soreness, decreases in peak knee extensor torque and increases in CK activity in the 24 and 48 h after the low or high volume bout of EIMD. There was no significant differences between the groups in perceived muscle soreness and peak knee extensor torque in the days after Bout 1, although the high volume group did show higher perceptions of muscle soreness and greater decrements in knee extensor torque compared to the low volume group. Creatine kinase responses in the high volume group were greater than those in the low volume group at both 24 and 48 h after Bout 1. This is consistent with previous research (Nosaka et al., 2001b; Brown et al., 1997; Howatson et al., 2007) and suggests that greater changes in muscle membrane permeability are evident after a more intensive bout of muscle-damaging exercise (Boros-Hatfaludy et al., 1986).

In agreement with studies investigating the repeated bout effect (Byrnes et al., 1985; Newman et al., 1987; Nosaka & Clarkson, 1995; Hortobagyi et al., 1998), the symptoms of EIMD were attenuated in the days after a second bout of muscle-damaging exercise. This reduction occurred regardless of whether participants performed an initial bout of low or high volume squatting exercise. This concurs with existing studies (Clarkson & Tremblay, 1988; Brown et al., 1997; Nosaka et al., 2001b; Chen et al., 2003; Chen & Nosaka, 2006; Howatson et al., 2007) to provide further evidence that a low volume of resistance exercise protects the muscle against a high volume of resistance exercise performed two weeks later.

As expected and in agreement with results from Chapters 3 – 5, the physiological, metabolic and perceptual responses during fixed-intensity running were heightened

as a result of inducing muscle damage. The findings were not significantly different between the groups, which suggests that similar alterations to fixed-intensity running occur irrespective of whether the initial bout of EIMD is of low or high volume. However, this study reaffirms that the detrimental effects of muscle-damaging exercise on fixed-intensity running are reduced after a repeated bout (see Chapter 5). Moreover, the protective effect of low volume muscle-damaging exercise against high volume EIMD is also transferable to treadmill running. That is, the responses after the repeated bout of high volume EIMD were reduced independent of whether this was preceded by low or high volume muscle-damaging exercise.

The mechanism responsible for the RBE is still an area of debate; the lower magnitude of muscle damage and its subsequent impact on fixed-intensity running could be centrally or peripherally orientated, or a combination of both. It has been suggested that unaccustomed exercise damages a pool of stress-susceptible sarcomeres, which upon recovery are replaced by stronger sarcomeres that are more resistant to a second bout of EIMD (Armstrong et al., 1983; Byrnes et al., 1985; Newham et al., 1987; McHugh et al., 1999a; McHugh, 2003). However, the initial bout does not have to cause extensive muscle damage to confer protection (McHugh et al., 1999a). If weak sarcomeres were still intact after the low volume bout of squatting, then they would have been damaged during the more strenuous high volume bout. However, CK response after the second bout of high volume squatting was reduced by the initial low volume bout. Therefore, in agreement with Nosaka et al. (2001b), it is unlikely that the RBE was exclusively due to the removal and replacement of weak sarcomeres. Clarkson and Tremblay (1998) suggested that the initial stress provided a stimulus to strengthen the muscle cell membrane against further injury. The reduction in CK suggests that a cellular adaptation, such as

improved muscle membrane integrity, might have occurred after the initial bout of low volume squatting exercise (Howatson et al., 2007). However, blood concentrations of intramuscular proteins reflect leakage from the muscle after EIMD and clearance from the blood (Warren et al., 1999). Vincent and Vincent (1997) postulated that trained individuals have an efficient mechanism for clearing CK from the blood. Therefore, that participants were accustomed to lower limb eccentric exercise after the initial bout, the lower CK values were possibly due to a greater CK clearance capacity.

The protective effect of low volume muscle-damaging exercise against subsequent high volume exercise could also be due to changes in motor unit recruitment. Using EMG, studies have observed a 10 – 20% reduction in MF during a second bout of high volume EIMD (Chen, 2003; Howatson et al., 2007), which suggests that a reduction in MF reflects a shift in muscle fibre recruitment from type II to type I (Warren et al., 2000). Since type I fibres are more resistant to muscle damage (Friden et al., 1983), it is conceivable that a greater activation decreased the stress on type II fibres and protected the muscle against damage from the second squatting bout (Nosaka et al., 2001b; Chen, 2003; Howatson et al., 2007). Electromyography amplitude can also be increased after eccentric exercise (Komi & Buskirk, 1972; Hortobagyi et al., 1996a; 1996b), meaning the repeated bout redistributes the contractile stress over a greater number of muscle fibres (McHugh et al., 1999a; McHugh, 2003). However, Chen (2003) and Howatson et al. (2007) observed that EMG amplitude was unchanged during a repeated bout of EIMD, which suggests that an increase in motor unit recruitment during the second bout of EIMD might not have contributed to the attenuation of muscle damage.

This is the first study to confirm that EMG amplitude is increased during fixed-intensity running after EIMD. Muscle fibre recruitment was potentially increased in order to complete the same running speed; given that knee extensor strength had decreased by 10.4 – 11.3% and 15.5 – 17.1% after initial low and high volume EIMD, respectively. As sensations of pain in muscle are mediated through Group III and IV afferent fibres (Jones & Round, 1990), the increase in muscle soreness after the unaccustomed bout of squatting exercise might have also contributed to the increase in EMG amplitude. Disturbances to these fibres as a result of EIMD can reduce locomotor muscle contractility, which might have caused a compensatory increase in central motor command to ensure participants were able to produce the force required during fixed-intensity running (Kyrolainen et al., 2000; Marcora, 2009). Since there is a linear relationship between EMG activity and oxygen uptake (Bigland-Ritchie & Woods, 1974), it is plausible that the observed increase in $\dot{V}O_2$ during fixed-intensity running after the initial bout of EIMD was reflective of an increase in EMG amplitude.

Alterations to stride pattern have also been attributed to an increase in fixed-intensity running $\dot{V}O_2$ after muscle-damaging exercise (see Chapters 3 and 5). Indeed, in line with Chapters 3 and 5, this study found that increases in stride frequency and decreases in stride length occurred alongside increases in $\dot{V}O_2$, which suggests that participants altered their normal stride to limit the discomfort associated with muscle soreness (Braun & Dutto, 2003). However, the attenuation of muscle soreness and maintenance of muscle function after the repeated bout enabled the preservation of stride pattern and motor unit activation during fixed-intensity running, and possibly explains why $\dot{V}O_2$ remained unchanged.

Alongside inhibiting neural drive to the muscle, group III and IV afferents are likely to have caused the increase in \dot{V}_E during fixed-intensity running at 24 and 48 h after EIMD. Group III and IV afferents are located in the blood vessels of the exercising muscle which are distended after muscle damage, stimulating a discharge from the afferent fibres that results in \dot{V}_E increasing (Hotta et al., 2006; Davies et al., 2008; 2011a; Twist & Eston, 2009). Moreover, it is likely that the muscle soreness experienced after the initial squatting bout provided an additional stimulus to drive \dot{V}_E during fixed-intensity running (Davies et al., 2009). Muscle soreness can activate nociceptors and mechanoreceptors, which subsequently increase \dot{V}_E response (Mense, 1977; Duranti et al., 1991; Gleeson et al., 1995). Ventilation was unaltered after the repeated bouts of squatting exercise and the protective effect was also evident after the bout of low volume resistance exercise. It is proposed that the initial bout strengthened the sarcolemma that subsequently increased muscle fibre integrity during the second bout. This cellular adaptation might have prevented the stimulation of afferent fibres that increased \dot{V}_E after Bout 1.

That \dot{V}_E remained unchanged after the repeated bout could explain the concomitant reduction in RPE during fixed-intensity running. Effort perception during exercise involves a combination of sensory cues that are derived from many different sources (Hampson et al., 2001). It is possible that feelings of breathlessness (as evidenced through the increase in \dot{V}_E) provided a central cue to determine RPE (Davies et al., 2009) during fixed-intensity running after the initial bout of squatting exercise. Likewise, the muscle soreness experienced in the knee extensors might have also provided a peripheral cue that altered the sense of effort (Jameson & Ring, 2000; Davies et al., 2009; Twist & Eston, 2009). Marcora (2009) proposed that RPE is

generated centrally by forwarding neural signals from the motor to the sensory areas of the cerebral cortex. Arguably, the increased central motor command required to produce the same baseline running speed after Bout 1 was responsible for the elevation in RPE (Scott et al., 2003; Elmer et al., 2010). If this was the case, then the lower EMG activity during fixed-intensity running after the repeated bout might also explain why RPE was unchanged.

The increase in [La] during fixed-intensity running after the initial bouts of squatting exercise supports results from Chapters 4 and 5. These findings are consistent with the observed increase in EMG activity that reflects an increase in the recruitment of non-damaged, more glycolytic, type II fibres in order to maintain pre-damage force capacity (Braun & Dutto, 2003). Alternatively, an increased efflux of lactate from the muscle might have occurred due to the increased muscle membrane permeability after the first bout of EIMD (Gleeson et al., 1995). It is also plausible that the neural and/or peripheral adaptations that explain the attenuated EMG activity during fixed-intensity running and the reduction in symptoms of EIMD contributed to the unaltered [La] response during the fixed-intensity running after the repeated bout of squatting exercise.

Running time-trial performance was impaired after the initial bout of resistance exercise. The time to complete 3 km was increased by 7% and 9% after low and high volumes of resistance exercise, respectively. This corresponds with prior studies, whereby distance covered during cycling (Twist & Eston, 2009; Burt & Twist, 2011) and running (Marcora & Bosio, 2007) time-trials has been decreased by 4 – 6% after EIMD. However, this is the first study to demonstrate that the detrimental effects of EIMD on running time-trial performance are attenuated after a second

bout. This study also shows that time-trial running after the repeated bout of high volume squatting was protected by a preceding low volume bout.

Studies investigating the effects of EIMD on force estimation have shown that humans perceive a higher effort for the same force and produce less force for the same perception of effort (Miles et al., 1997; Weerakkody et al., 2003; Proske et al., 2004). In the current study, and in support of Marcora and Bosio (2007), participants ran at a slower speed despite their RPE being similar to pre-damage during the time-trial after Bout 1. It is speculated that muscular pain incurred as a result of the initial bout of squatting exercise altered the sense of effort during the time-trial, which resulted in participants adopting a slower speed and the subsequent lower $\dot{V}O_2$, \dot{V}_E , HR and [La] responses. Nonetheless, the attenuation of muscle soreness as a result of the RBE would have enabled the sense of effort to be maintained during the time-trial after Bout 2, which subsequently led to the preservation of time, speed, $\dot{V}O_2$, HR and [La] responses.

Contradicting findings from Khan et al. (2011) suggest that pain does not influence sense of effort during exercise. Likewise, Marcora et al. (2008) found that 40 minutes after muscle-damaging exercise, increases in RPE during cycling occurred despite no evidence of muscle soreness. They suggested that the sense of effort was determined by an increase in central motor command and not muscle pain. The premature termination of fixed-intensity time-to-exhaustion trials after EIMD have also been attributed to an increase in central motor command, which enabled participants to exercise at the same relative workload after EIMD (Doncaster & Twist, 2012). In the current study, if participants ran at the same pre-damage speed during the time-trial after EIMD, it is possible that an increase in central motor command

would have prevented them from completing the task. Therefore, due to the impaired muscle function after Bout 1, participants reduced their speed to a tolerable running speed to complete the 3 km. Accordingly, the preservation of muscle function after Bout 2 would have maintained central motor command and subsequently time-trial performance.

Alternatively, Marcora and Bosio (2007) hypothesised that the inflammatory response associated with EIMD would reduce running time-trial performance. Interleukin (IL)-1 is a primary mediator of the muscle's inflammatory response to EIMD (Tidball, 1995) and once evident in the muscle is speculated to enter circulation where it can have a direct effect on the brain (Carmichael et al., 2005). Studies have shown that increased levels of IL-1 in the brain causes symptoms of fatigue in human participants (Rinehart et al., 1997; Omdal & Gunnarsson, 2005). Furthermore, Carmichael et al. (2005) observed that decrements in endurance performance after EIMD in mice coincided with elevations in IL-1 in the cortex and cerebellum regions of the brain. The attenuation of inflammatory markers has been observed after a repeated bout of muscle-damaging exercise (Pizza et al., 1996). Therefore, it is possible that an adaptation that prevented the disruption of muscle fibres and the subsequent inflammatory response reduced the negative effect of EIMD on time-trial performance.

Despite this study being the first to show that the detrimental effects of EIMD on running time-trial performance are attenuated after repeated muscle-damaging exercise, the findings are limited to short-term (3 km) endurance performance and recreationally active participants. Given this, future research should consider investigating the effects of repeated bouts of muscle-damaging exercise during long

distance running (> 5 km) among elite endurance runners. It is accepted that there are further limitations with the time-trial protocol. Indeed, pacing strategy during the time-trial was not assessed and given that pacing strategy is altered as a result of EIMD (Burt & Twist, 2011); future research should investigate if changes to pacing during time-trial running are still evident after a repeated bout of EIMD. There are also concerns with the standard deviations of the end RPE data (see Figure 6.2 f). Indeed, for 3 of the 4 trials, the spread of the end RPE data are greater than 20. The RPE scale is relatively narrow (6 – 20), which might lend itself to such problems when treated as an interval level of measurement. Furthermore, session RPE could have been measured to provide an overall perception of effort during the time-trial, as opposed to asking participants to rate their RPE in the last 50 m of the time-trial.

In conclusion, Chapter 5 demonstrated that the effects of EIMD on fixed-intensity endurance exercise are attenuated after a repeated bout of muscle-damaging exercise performed two weeks later. Accordingly, this study examined if a low volume bout of muscle-damaging exercise performed two weeks prior to a high volume bout of the same exercise provided the same protective effect on endurance running exercise. To address this aim, participants completed bouts of fixed-intensity and 3 km time-trial running before and 24 – 48 h EIMD comprising low volume or high volume squats. To ascertain the RBE, participants repeated the baseline measurements, the high volume squats and the same follow-up testing two weeks later. This investigation reaffirmed that EIMD causes alterations to fixed-intensity and time-trial running performance. However, a low volume bout of muscle-damaging exercise was shown to provide protection against a high volume bout performed two weeks later. Furthermore, in the days after this repeated bout of EIMD, alterations to fixed-intensity and 3 km time-trial running were attenuated. From a practical

standpoint, where endurance athletes are contemplating concurrent endurance and resistance exercise to enhance performance, this study shows that performing a lower volume of resistance exercise preconditions the muscle to withstand higher bouts of muscle-damaging exercise and its detrimental effects on fixed-intensity and time-trial running.

CHAPTER 7

CONCLUSIONS

7.1 Limitations

7.1.1 Participant training status

Since the participants recruited for each study comprised recreationally active young males whom engaged in 2 – 3 sessions of physical activity per week, the findings can only be extrapolated to those with matching physiological characteristics. Given that the responses to EIMD appear to be different between trained and untrained (Falvo et al., 2007; Hackney et al., 2008) and adults and children (Marginson et al., 2005), the application of the findings presented in this thesis to other populations would be inappropriate.

7.1.2 The lack of underpinning mechanisms to explain findings

The significance of this thesis is limited by a lack of mechanistic experimentation to explain the effects of EIMD on endurance performance. Evidence that EIMD had occurred after squatting exercise was inferred using indirect markers of muscle damage. More complex histological analysis was required to confirm that damage has occurred at the site of the muscle. Furthermore, this thesis demonstrates that the effects of EIMD on endurance performance are attenuated after a repeated bout of muscle damage. However, the mechanisms used to explain this phenomenon have been based on neural and peripheral adaptations that have been confirmed or suggested in previous studies. Further blood, muscle biopsy and EMG analysis was needed to confirm the hypotheses put forward. Finally, some explanations of the findings have been attributed to concomitant alterations in other measured variables.

For example, increases in $\dot{V}O_2$ during sub-maximal running post-EIMD were attributed to changes in stride length and frequency. Despite the changes in these variables showing good agreement, this does not imply cause-and-effect.

7.1.3 Exercise-induced muscle damage protocol

To incur EIMD, participants were required to perform squats at a resistance corresponding to 80% of body mass. Whilst, this resistance was effective in inducing symptoms associated with EIMD, there are limitations surrounding its relevance to resistance training. Indeed, a muscle-damaging protocol that incorporated a range of lower limb resistance exercises would have replicated typical resistance training; it is unlikely that individuals would perform 100 repetitions of one exercise. The protocol also failed to account for inter-individual differences. For example, a resistance corresponding to 80% body mass for one participant might have been heavy, but for another individual might have been moderate. Alternatively, a resistance comprising %MVC or %1RM would have accounted for any inter-individual responses.

7.2 Future directions

7.2.1 Effects of muscle-damaging exercise on endurance exercise in the well-trained

Whilst a large amount of research examining the effects of EIMD on endurance performance has been conducted in recreationally active adults (Gleeson et al., 1995; Braun & Dutto, 2003; Chen et al., 2007b; 2009; Davies et al., 2009; Twist & Eston, 2009; Burt & Twist, 2011), it is not known if the same responses are seen amongst the well-trained. Research has shown that responses to EIMD are dependent on training status. For example, Hackney et al. (2008) demonstrated that symptoms of EIMD were less pronounced in resistance trained males compared with

untrained males after muscle-damaging exercise. Therefore, an interesting future study would be to examine if the effects of EIMD on physiological, metabolic and perceptual responses during endurance exercise are similar between untrained and well-trained athletes.

7.2.2 Effects of repeated bouts of muscle-damaging exercise on athletic performance

Despite the well-established body of studies advocating that the effects of EIMD are negated after a repeated bout of muscle-damaging exercise, a common limitation among RBE research is its lack of application to athletic performance. As far as the author is aware, Chapters 5 and 6 describe the only two studies to examine the RBE in an athletic performance context. It was observed that the detrimental effects of muscle damage on sub-maximal and time-trial running performance are attenuated in the days after a repeated bout of EIMD. However, EIMD has been shown to limit power (Sargeant & Dolan, 1987; Byrne & Eston, 2002b; Twist & Eston, 2007), sprint performance (Twist & Eston, 2005; Highton et al., 2009) and agility (Highton et al., 2009). Therefore, it remains to be seen if these alterations are still apparent in the days after a second bout of muscle-damaging exercise.

Prior research has shown that the attenuation of symptoms associated with muscle damage after a repeated bout can last between two and six months (Byrnes et al., 1985; Nosaka et al., 2001a). However, the length of protection on athletic performance is currently not known. Future research should look to investigate how long the RBE on endurance performance lasts.

7.2.3 Investigation into the cross-over effect on endurance exercise

It appears that EIMD in one limb serves to protect against repeated muscle-damaging exercise in the opposite limb. Referred to as the “cross-transfer effect”, two studies (Howatson & van Someren, 2007; Starbuck & Eston, 2012) have both shown that a prior bout of muscle-damaging exercise in one arm reduces the symptoms of EIMD when a repeated bout of muscle damage is performed in the opposite arm two weeks later. An interesting future investigation would be to investigate if the effects of EIMD on the physiological, metabolic and perceptual responses during single leg cycling protect against EIMD during subsequent single leg cycling performance in the opposite leg. A study of this kind could have implications for endurance athletes with a single limb injury.

7.2.4 Nutritional strategies to alleviate the effects of exercise-induced muscle damage on endurance exercise

The results of this thesis clearly demonstrate that symptoms of EIMD occur after an unaccustomed bout of heavy squatting exercise. Furthermore, these symptoms were shown to impair sub-maximal and time-trial endurance performance. However, nutritional interventions that alleviate the symptoms of EIMD have long been of interest to researchers. For example, milk (Cockburn et al., 2012), branched chain amino acids (Howatson et al., 2012) and cherry juice (Howatson et al., 2010; Bowtell et al., 2011) have been found to reduce muscle soreness, inflammation and CK and recover muscle function after EIMD. However, whether any of these nutritional aids can attenuate the detrimental effects of muscle damage on endurance performance remains to be elucidated.

7.3 Main findings

7.3.1 The effects of exercise mode on the physiological responses to exercise-induced muscle damage

Despite a different time course, sub-maximal $\dot{V}O_2$ is increased after muscle damaging exercise during both cycling (Chapter 3) and running (Chapters 3 – 6). Across each study, $\dot{V}O_2$ during sub-maximal running was elevated at 24 and 48 h after EIMD, whilst cycling $\dot{V}O_2$ (in Chapter 3) was only increased after 48 h. The increased $\dot{V}O_2$ response during sub-maximal running is attributed to changes in running stride pattern (as shown in Chapters 3, 5 and 6) and possibly a decreased ability to use the SSC (as shown in Chapter 5). The unexpected increase in cycling $\dot{V}O_2$ might be due the increase in motor unit recruitment in order to produce the same pre-damage force. Indeed, Chapter 6 demonstrates that increases in motor unit recruitment (measured using EMG) occur alongside increases in $\dot{V}O_2$ during sub-maximal endurance exercise after EIMD.

The findings of this thesis also extend the growing body of research that demonstrates that \dot{V}_E during sub-maximal endurance exercise is increased after EIMD. Across each of the four studies, \dot{V}_E was elevated during sub-maximal endurance exercise after muscle-damaging exercise. Moreover, this finding occurred independent of the mode of endurance exercise investigated. However, as with $\dot{V}O_2$ response, \dot{V}_E during sub-maximal running was increased at 24 and 48 h after EIMD (Chapters 3 – 6), whilst \dot{V}_E during sub-maximal cycling was only elevated at 48 h (Chapter 3). It is proposed that damage to the musculature provokes a discharge from Group III and IV afferent fibres resulting in the increase in \dot{V}_E during sub-maximal endurance exercise. The impaired ability to use the SSC after EIMD is

thought to be due to the activation of afferent fibres (Avela et al., 1999). Thus, the activation of Group III and IV afferents inhibiting the SSC as a result of EIMD might have caused the earlier elevation in \dot{V}_E during running. Muscle soreness also provokes an increased \dot{V}_E response through activating nociceptive muscle afferents (Duranti et al., 1991). Thus, the heightened muscle soreness 48 h after EIMD might have been responsible for the increase in \dot{V}_E during cycling.

7.3.2 The effects of muscle damage on resting metabolic rate, exercise metabolism and excess post-exercise oxygen consumption

The effects of EIMD have been shown to increase oxidative metabolism both before (rest) and during endurance exercise. However, its effect on recovery (EPOC) from endurance exercise had not been previously investigated. In the presence of EIMD, $\dot{V}O_2$ was increased before (resting metabolic rate), during and in the 30 minutes after sub-maximal running (EPOC). Resting metabolic rate was suggested to be elevated due to the increased energy required for the breakdown and repair of damaged muscle fibres. The increased oxygen cost during sub-maximal running informs that for a given exercise intensity participants are exercising harder whilst muscle damaged. Indeed, the 4 – 5% increase in $\dot{V}O_2$ during sub-maximal running (as shown in Chapter 4) indicated that participants were exercising at 89 – 90% $\dot{V}O_{2\max}$, as opposed to 85% $\dot{V}O_{2\max}$ before EIMD. The increase in $\dot{V}O_2$ during running post-EIMD is also likely to be responsible for the elevation in EPOC. Chapter 4 examined the effect of EIMD on EPOC after endurance exercise, whereby total $\dot{V}O_2$ was increased by 4 – 6% during recovery from sub-maximal running after EIMD. Other mechanisms associated with the increase in EPOC include elevations in blood lactate concentration, minute ventilation, heart rate and resting metabolic rate. Blood

lactate at the end of sub-maximal running was 6 – 14% higher in the presence of muscle damage and the increase in EPOC might have been required to reconvert lactate to glycogen (Gaesser & Brookes, 1984; Bangsbo et al., 1991). Alternatively, EPOC might have increased to facilitate the elevated \dot{V}_E response due to the activation of Group III and IV afferent fibres after muscle damage. Recovery heart rate was also increased following sub-maximal running post-EIMD and stress associated with the movement of sore muscles might have secreted catecholamines causing the increase in EPOC (Moreau et al., 1995). Moreover, the 11.8 – 13.2% increase in resting metabolic rate after EIMD might have had a residual effect on EPOC after the sub-maximal running exercise.

7.3.3 The effects of repeated bouts of muscle-damaging exercise on endurance performance

It is well documented that the symptoms of muscle damage are reduced after a repeated bout of EIMD. However, it was not known if this protective adaptation was transferable to endurance performance. Physiological, metabolic, perceptual and kinematic responses during sub-maximal and time-trial running were measured before and after (24 – 48 h) an initial bout of EIMD. Two weeks later, when symptoms associated with Bout 1 had disappeared; responses during the same sub-maximal exercise were measured again after a repeated bout of the same muscle-damaging exercise. The results informed that while novel muscle-damaging exercise was detrimental to endurance performance, the muscle underwent an adaptation that attenuated these changes in the days after the repeated bout. This protective adaptation was also shown after a low volume bout of muscle-damaging exercise. Indeed, a bout of low volume EIMD (50 squats at 80% body mass) protected the

muscle against damage after a high volume bout (100 squats at 80% body mass) performed two weeks later. Furthermore, Chapter 6 shows that the protective effect of low volume muscle-damaging exercise against high volume EIMD is transferable to fixed-intensity and time-trial running. It is posited that an interaction of neural and peripheral adaptations occurred to protect the muscle from the effects of EIMD on endurance performance.

7.4 Summary

The overall aim of this thesis was to investigate the effects of EIMD on endurance performance. This objective was addressed through four empirical studies, all of which contribute to the existing body of research into EIMD. For the first time, physiological, metabolic and perceptual responses to different modes of endurance exercise were examined after muscle-damaging exercise. While, responses were altered in both cycling and running as a result of EIMD, the time course of these responses were mode specific with running impacted at 24 and 48 h after EIMD and cycling at only 48 h. Previous research and results from Chapter 3 inform that oxidative metabolism, in the presence of EIMD, is increased at rest and during endurance exercise. Chapter 4 extends these findings to confirm that EIMD also increases oxygen uptake during recovery from endurance exercise. It has long been shown that symptoms of EIMD are reduced after a repeated bout of muscle-damaging exercise. For the first time, alterations to physiological, metabolic, perceptual and kinematic responses during sub-maximal running after an initial bout of EIMD are attenuated in the days after a repeated bout of EIMD. Furthermore, in Chapter 6, these findings were extended to running time-trial performance. Chapter 6 also demonstrated that performing an initial bout of low volume EIMD protected the

muscle against a repeated bout of high volume EIMD and the detrimental effects on endurance performance. Finally, from a practical standpoint, individuals contemplating resistance exercise for the first time should be considerate of the consequences EIMD can have on endurance exercise. Thereafter, as the latter two studies demonstrate, the muscle will be preconditioned to withstand repeated bouts of muscle-damaging exercise and its negative effects on endurance performance.

CHAPTER 8

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CHAPTER 9

APPENDICES

APPENDIX 1

Participant information sheet (taken from study 4)

Title of the study: Does a lower volume of muscle-damaging exercise confer protection against a higher volume of muscle-damaging exercise and the detrimental effects on endurance running?

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please feel free to ask the lead researcher if there is anything that is not clear or if you would like more information. Please take your time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of this study?

The aims of this study are to investigate whether a lower volume of muscle-damaging exercise can protect the muscle against a higher volume of muscle-damaging exercise and the detrimental effects exercise-induced muscle damage (EIMD) has on endurance running performance.

Why have I been chosen?

You have been asked to take part because you are from an endurance trained population who has had limited experience of lower limb resistance training.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You are then still free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

You will be required to attend 8 different exercise trials over a 5-week period. The first visit will require you to complete a maximal lactate threshold test in which you will be instructed to run to exhaustion. Additionally, a habituation session to familiarise you with the measurements of leg strength, perceived muscle soreness, and time-trial running will also take place. Forty eight hours later, you will complete a leg strength test, from which the electrical activity from two muscles in your quadricep will be recorded (Visit 2). Upon completion, you will then be randomly assigned to either a low volume or high volume muscle damage group. A week later you will return to complete baseline measurements (visit 3), which will consist a 5-minute bout of moderate intensity running, a 3 km running time-trial, measurements

of leg strength, perceived muscle soreness, a finger-prick sample of blood for creatine kinase content, followed by either a low (5 sets of 10 squats) or high (10 sets of 10 squats) volume bout of resistance exercise designed to develop symptoms of muscle damage. You will then return to the laboratory 24 (visit 4) and 48 hours (visit 5) later to complete the same procedures outlined at baseline, in the same order (except for the time-trial at 24 hours). Approximately two weeks later, when symptoms associated with the initial bout of muscle-damaging exercise have dissipated, you will repeat all testing procedures outlined at baseline, the high volume bout of muscle-damaging exercise (visit 6) and the same follow-up testing at 24 (visit 7) and 48 hours (visit 8).

You will be required not to consume food or drink in the 2 hours prior to any exercise testing, and not to take part in strenuous exercise in the 24 hour period before any exercise testing. In addition no alcohol must be consumed in the 24 hour period before any exercise testing.

What are the possible disadvantages and risks of taking part?

You will experience a short bout of muscle soreness as a consequence of the resistance exercise. This will be most evident approximately 48 hours following the muscle-damaging exercise, after which symptoms will ease and will have disappeared by approximately one week later. These symptoms are common in all exercising populations, particularly following a bout of unaccustomed exercise and have no lasting effect (please read the 'protocol for the management of exercise-induced muscle damage' leaflet for further advice on how to manage your muscle soreness).

What are the possible benefits of taking part?

Participation in this study includes the direct assessment of your aerobic fitness level, which you can subsequently use to prescribe intensities for training. Additionally, you should be protected from symptoms of muscle damage for approximately six months following other subsequent bouts of muscle-damaging exercise.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Applied Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential, only the lead researcher and his supervisors will have access to such information. All of your raw data will be stored in a locked filing

cabinet which will only be accessed by the lead researcher. All electronic formats of the data will be coded to ensure your anonymity.

What will happen to the results of the research study?

The results will be written up into a report as part of the lead researcher's PhD thesis. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising and funding the research?

The Department of Sport and Exercise Sciences is funding the research project. Dean Burt, a PhD student in the Department of Sport and Exercise Sciences at the University of Chester will be involved in organising and carrying out the study.

Who may I contact for further information?

If you would like more information about the research before you decide to take part, please contact:

Dean Burt at

APPENDIX 2

Pre-test Health Questionnaire

(Please note that this information will remain confidential)

Name:..... Date of Birth:..... Age:.....

Resting Blood Pressure (mmHg)...../..... Resting Heart Rate (b.min⁻¹).....

Please answer these questions truthfully and completely. The purpose of this questionnaire is to ensure that you are fit and healthy enough to participate in this research project.

	YES	NO
1. Have you ever experienced a serious illness or accident?	<input type="checkbox"/>	<input type="checkbox"/>
If yes, please provide details.		
.....		

	YES	NO
2. Have you consulted your doctor in the last 6 months?	<input type="checkbox"/>	<input type="checkbox"/>
If yes, please provide details		
.....		

3. Do you suffer, or have you suffered from any of the following:

	YES	NO
Asthma	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
Bronchitis	<input type="checkbox"/>	<input type="checkbox"/>
Epilepsy	<input type="checkbox"/>	<input type="checkbox"/>
High blood pressure	<input type="checkbox"/>	<input type="checkbox"/>

	YES	NO
4. Is there a history of heart disease in your family?	<input type="checkbox"/>	<input type="checkbox"/>

	YES	NO
5. As far as you are aware are you suffering from any infectious skin diseases or blood infections i.e., Hepatitis B, HIV?	<input type="checkbox"/>	<input type="checkbox"/>
If yes, please provide details.		
.....		

	YES	NO
6. Are you currently taking any medication?	<input type="checkbox"/>	<input type="checkbox"/>
If yes, please provide details		
.....		

	YES	NO
7. Are you suffering from any disease that inhibits the sweating process?	<input type="checkbox"/>	<input type="checkbox"/>

	YES	NO
8. Is there anything to your knowledge that may prevent you from participating in the testing that has been outlined to you?	<input type="checkbox"/>	<input type="checkbox"/>

If yes, please provide details.

.....

Your recent condition	YES	NO
Have you had any kind of illness or infection in the last 2 weeks?	<input type="checkbox"/>	<input type="checkbox"/>

Evaluate your diet over the last 2 days. **POOR AVERAGE GOOD EXCELLENT**

	YES	NO
Have you consumed alcohol in the last 24 hours?	<input type="checkbox"/>	<input type="checkbox"/>

	YES	NO
Have you eaten in the last 2 hours?	<input type="checkbox"/>	<input type="checkbox"/>

If yes, please provide details

.....

	YES	NO
Are you currently engaging in regular physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

If yes, please describe below

.....

	YES	NO
Have you ever done lower body resistance exercise?	<input type="checkbox"/>	<input type="checkbox"/>

If yes, please provide details on the last time?

.....

	YES	NO
Have you exercised in the last 2 days?	<input type="checkbox"/>	<input type="checkbox"/>

If yes, please describe below

.....

Persons will not be permitted to take part in any experimental testing if they:

- Have a known history of medical disorders (i.e. hypertension, heart or lung disease)
- Have a fever, suffer from fainting or dizzy spells
- Are currently unable to train because of a joint or muscle injury
- Have had any thermoregulatory disorder
- Have gastrointestinal disorder
- Have a history of infectious diseases (i.e. HIV or Hepatitis B)
- Have, if pertinent to the study, a known history of rectal bleeding, anal fissures, haemorrhoids, or any other similar rectal disorder.

My responses to the above questions are true to the best of my knowledge and I am assured that they will be held in the strictest confidence.

Name: (Participant).....Date:.....

Signed: (Participant).....

Name: (Researcher).....Date:.....

Signed (Researcher):.....

APPENDIX 3

Informed consent form

Title of project: Does a lower volume of muscle-damaging exercise confer protection against a higher volume of muscle-damaging exercise and the detrimental effects on endurance running?

Name of researcher: Dean Burt

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;
2. I am free to withdraw from the project at any time without any disadvantage;
3. The data will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage and coded to endure anonymity;
4. I am aware that I may experience a short, bout of mild muscle soreness following the squatting exercise protocol.
5. The results of the project may be published but my anonymity will be preserved.

I agree to take part in this project.

.....
(Signature of participant)

Date/...../.....

APPENDIX 4

Protocol for the management of exercise-induced muscle damage

It is likely that you may experience a short bout of muscle soreness during your participation in this study. This will be most evident approximately two days following the resistance exercise, after which symptoms of soreness will subside and will have disappeared after one week. Please note that this response is quite normal in people who commence new exercise routines or suddenly increase the volume or intensity of their training.

Although you are asked to refrain from using any methods to alleviate the symptoms of muscle damage, the following details may be of use in the days following muscle-damaging exercise to assist in the management of the symptoms.

- 1) Avoid participation in strenuous exercise, other than that required as part of this study.
- 2) Immediately following and at 24 and 48 hours following the resistance exercise, the muscles that have been damaged are likely to be at their sorest and weakest (please note that this will be about 80-85% of their normal strength). You should be mindful that activities requiring use of the legs (e.g. walking, running, descending/climbing stairs) may be slightly more difficult, but not to the point that will affect your everyday activities.
- 3) Avoid the use of non-steroidal anti-inflammatory medication (e.g. ibuprofen), ice treatment or compression garments, this will not only invalidate the study but there is also no clear evidence that the use of such interventions can speed the recovery process.

APPENDIX 5

Ethical approval for Study 1

University of Chester
Faculty of Applied and Health Sciences
Research Ethics Committee

Dean Burt
Sport and Exercise Science Department CTW 507
University of Chester
Parkgate Road
Chester
CH1 4BJ

2nd July 2009

Dear Dean,

Study title: The effects of exercise-induced muscle damage on fixed-intensity running and cycling performance.

FREC reference: 318/09/DB/SES

Version number: 1

Thank you for sending the above-named application to the Faculty of Applied and Health Sciences Research Ethics Committee for review.

The application has been considered by the Faculty Research Ethics Committee.

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation.

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Response to the Committee	1	June 2009
Pre-test Health Questionnaire	2	June 2009
Informed Consent form	2	June 2009

With the Committee's best wishes for the success of this project.

Yours sincerely,

Mohammed Saeed

Chair, Faculty Research Ethics Committee

Enclosures Standard conditions of approval.

c.c. Supervisor
FREC Representative

Ethical approval for Studies 2 and 3

University of Chester
Faculty of Applied and Health Sciences
Research Ethics Committee

Dean Burt
c/o Department of Sport and Exercise Sciences
Faculty of Applied and Health Sciences
University of Chester
Chester campus

29 April 2010

Dear Dean

Study title: **The effects of repeated bouts of muscle damaging exercise on sub-maximal endurance performance and post-exercise oxygen consumption**

FREC reference: **400/10/DB/SES**

Version number: **2**

Thank you for sending the above-named application to the Faculty of Applied and Health Sciences Research Ethics Committee for review.

The application has been considered on behalf of the Committee by Mohammed Saeed as Lead Reviewer and reported to the Faculty Research Ethics Committee.

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation.

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	March 2010
List of references	1	March 2010
Summary CV of applicant	-	March 2010
Participant information sheet	2	April 2010
Consent form	1	March 2010

Protocol for managing DOMS	1	March 2010
Risk assessment forms	1	March 2010
Pre-test health questionnaire	2	April 2010
Schematic of research	2	April 2010
Response to FREC request for clarification/additional information	1	April 2010

With the Committee's best wishes for the success of this project.

Yours sincerely,

Prof. Cynthia Burek

Chair, Faculty Research Ethics Committee

Enclosures Standard conditions of approval.

c.c. Supervisor
FREC Representative

Ethical approval for Study 4



University of
Chester

**Faculty of Applied Sciences
Research Ethics Committee**

Tel 01244 511740
Fax 01244 511302
frec@chester.ac.uk

Dean Burt
Department of Sport and Exercise Science
University of Chester
Parkgate Road
Chester
CH1 4BJ

10th August 2011

Dear Dean,

Study title: Does a lower volume of muscle-damaging exercise confer protection against a higher volume of muscle-damaging exercise and the detrimental effects on endurance running?

FREC reference: 597/11/DB/SES

Version number: 1

Thank you for sending your application to the Faculty of Applied Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	July 2011
Appendix 1 – List of References	1	July 2011
Appendix 2 – C.V. for Lead Researcher	1	July 2011
Appendix 3 – Participant Information Sheet	1	July 2011
Appendix 4 – Participant Consent Form	1	July 2011
Appendix 5 – Information Sheet – Protocol for the management of exercise-induced muscle damage.	1	July 2011
Appendix 6 – Risk Assessment Form	1	July 2011

Appendix 7 – Pre-test Health Questionnaire	1	July 2011
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With the Committee's best wishes for the success of this project.

Yours sincerely,

Simon Alford

Chair, Faculty Research Ethics Committee

Enclosures: Standard conditions of approval.

C.c. Supervisor
FREC Representative

APPENDIX 6

Tukey test modified for repeated measures

The post hoc Tukey test modified for repeated measures is described by Stevens (2002) and has been used successfully in previous research to determine where significant differences occurred before and after muscle-damaging exercise (Davies et al., 2009).

In brief, two means were declared significantly different at the 0.05 alpha level if the following occurred:

$$\text{Mean 1} - \text{Mean 2} > q \sqrt{\text{MSres}/n}$$

where q is the studentized range statistic (computed from the number of means being compared and the error degrees of freedom), MSres is the mean square error and n is the number of participants.

A working example for muscle soreness:

$q = 4.56$ (based on number of means compared being 6 and the error degrees of freedom being 16), $\text{MSres} = 0.679$ (based on the Mean Square error value), $n = 9$ (number of participants).

$$4.56 \sqrt{0.679/9} = 1.25$$

From this, any two means with a difference greater than 1.25 were significantly different at the 0.05 alpha level. Therefore, as shown in the table below, there is a significant difference within each bout (i.e. Baseline vs 24h; Baseline vs 48h) and between each bout (i.e. Bout 1 24h vs Bout 2 24h; Bout 1 48h vs Bout 2 48h).

Table. Muscle soreness responses after repeated bouts of muscle damage

Bout 1			Bout 2		
Baseline	24 h	48 h	Baseline	24 h	48 h
0.2 ± 0.5	5.5 ± 1.6	6.2 ± 2.1	0.0 ± 0.1	2.1 ± 1.0	1.3 ± 0.9

APPENDIX 7

RATING OF PERCEIVED EXERTION

6	NO EXERTION AT ALL
7	
	EXTREMELY LIGHT
8	
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

Borg RPE scale (Borg, 1998)

Instructions for Borg's RPE scale

Whilst exercising, I want you to rate your perceived level of exertion. This rating should reflect how heavy and strenuous you perceive the exercise to be. Your perception of exertion should combine all sensations of strain and fatigue felt in your muscles and any feelings of breathlessness or aches in your chest. Try not to base your perceived exertion on any single factor, such as feelings of breathlessness, and instead provide an overall feeling of exertion that encompasses all sensations and feelings experienced during the exercise.

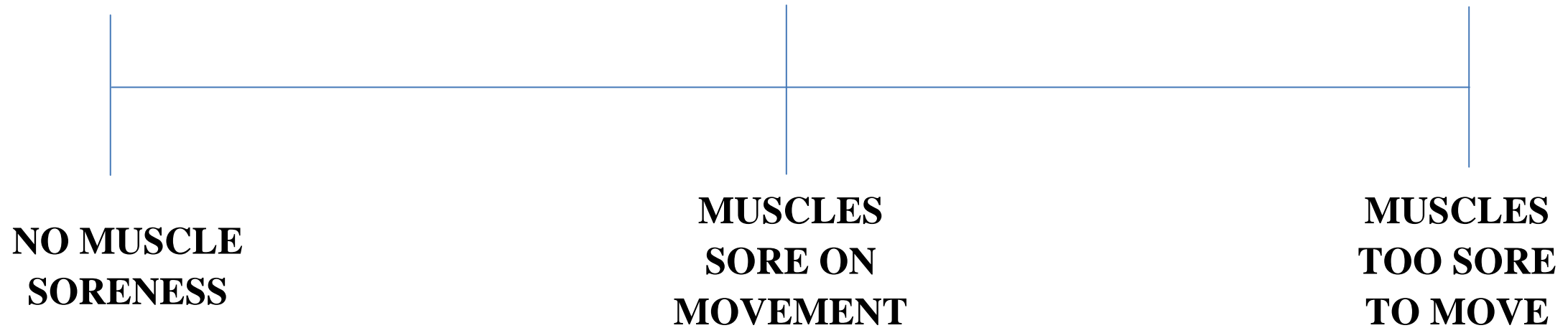
Looking at the rating scale, you will notice that it ranges from 6 to 20, where 6 corresponds to 'no exertion at all' and 20 refers to 'maximal exertion'. When required to do so, look at the scale and the corresponding written cues and point to a number that best describes your rating of perceived exertion. Please try to rate your feelings of exertion as honestly as possible, without thinking about what the actual physical load is. Remember it is your own feelings of exertion that are important, not how it compares to others. Please see below for example definitions of the RPE scale.

- 9 refers to 'very light' exercise. For a healthy individual, it is like walking slowly at their own pace for some minutes
- 13 on the RPE scale is 'somewhat hard' exercise, but still feels OK to continue.
- 17 'very hard' refers to very strenuous exercise. A healthy individual can still exercise, but they really have to push themselves. The exercise feels very heavy and the individual is very tired.
- 19 refers to 'extremely hard' exercise. For most individuals, this is the most strenuous exercise they have ever experienced.

Taken and adapted from Borg (1998)

APPENDIX 8

VISUAL ANALOGUE SCALE



Instructions for rating muscle soreness using the visual analogue scale

After squatting (with hands on your hips) to an approximate knee angle of 90 degrees, you will be asked to rate the perceived muscle soreness experienced in your quadriceps by moving a marker along a visual analogue scale (VAS).

The VAS is numbered from 0 to 10 (on the reverse of the scale, unseen by you), which correspond to three written cues. For instance, 0 corresponds to 'no muscle soreness', 5 signifies that the 'muscles is sore up movement' and 10 indicates that the 'muscle is too sore to move'. When required to do so, look at the VAS and move the marker along the continuum to a written cue that best describes your rating of perceived muscle soreness. Please rate your perception of muscle soreness as honestly as possible. Remember, this rating should reflect the overall pain felt in your quadriceps after squatting.